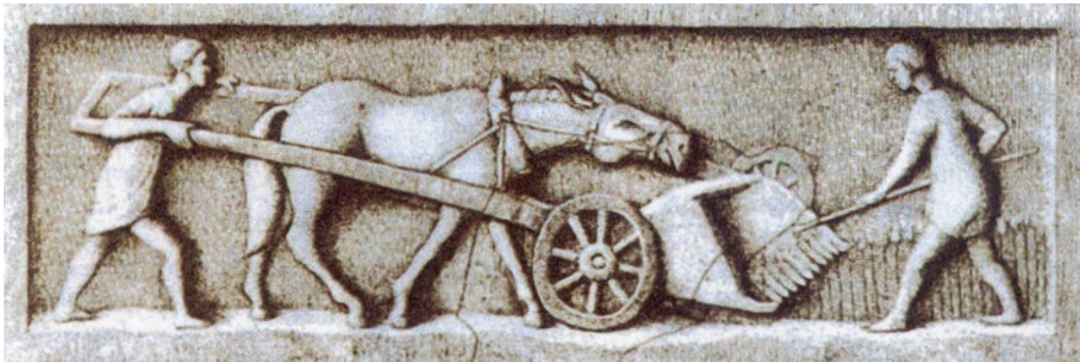


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# Columella

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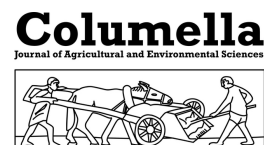
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## Agri-environmental impacts on yield formation of soybean crop

Rosnani ABD GHANI – Suhana OMAR – Elias EL CHAMI – Josepha EL CHAMI – Márton JOLÁNKAI

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**Abstract:** One of the most important leguminous crops that contributes to human alimentation and animal feed is soybean. The grain of the crop with its high nutritional value is an essential component for the food and feed industries worldwide. Grain yield of field crops highly depend on the agri-environmental conditions they are exposed to. The most influential factors are plant nutrition, plant protection and the influence of environmental, especially of biotic stresses. At the Department of Agronomy, Hungarian University of Agriculture and Life Sciences some agri-environmental impacts on grain yield of soybean crop have been studied in a replicated field trial. N application and various means of weed control was studied, and samples of grain yield were evaluated in accordance with the treatments. Apart from agronomic applications continuous observation and recording of game damages of the crop was implemented. The results obtained suggest, that N topdressing had positive, but no significant effect on the amount of grain yield, however the means of weed control resulted in an almost twofold yield improvement compared to the control. Rabbit bite damages were monitored during yield formation. The extent of game damage was consequent but not significant regarding crop yield.

**Keywords:** Soybean, grain yield, nitrogen, weed control, game damage

Received 21 April 2021, Revised 15 August 2021, Accepted 17 August 2021

### Introduction

Soybean (*Glycine max* L. Merr) is one of the most valuable leguminous crops grown worldwide for food and feed production due to its high nutritional properties. Soybean is a major protein source but has a considerable lipid content as well. The role of the crop is essential in human alimentation and in the production of animal feedstuffs. Yield and nutritional composition of soybean rely on environmental conditions, type of variety used, and agronomic practices including nutrient and weed management. Inefficient nutrient and weed management may cause a reduction in crop yield and nutritional value (Rotundo and Westgate, 2009). From among environmental factors abiotic and biotic stresses may profoundly influence crop performance and so yield formation (Miransari, 2016).

One of the important nutrients for soybean is nitrogen. Nitrogen (N) is vital for many processes in plants like chlorophyll and protein synthesis. The two main sources of N for soybean are biologically fixed N<sub>2</sub> and mineral N fertilizer (Salvagiotti et al., 2008). N fertilization must be provided if a deficiency in fixed N<sub>2</sub> occurs (Miransari, 2016). Many previous studies have been conducted on the N requirement for different soybean varieties in various areas on yield and seed composition. Wood et al., (1993) recorded a positive effect on grain yields of soybean occurred for treatment that used N fertilizer in different locations. The results of this work suggest that N fertilizer application is the best in a rising proposition. Taylor et al. (2005) reported the same finding that N application increased seed yield regardless of planting date, cultivar, or crop site.

Weed control is a very important manage-

Table 1: Experimental treatments and their abbreviations

Treatments	
N1	0 N
N2	200 kg N/ha
W1	Weedy
W2	Hand weeded
W3	Mechanically weeded

ment practice in soybean cultivation. Soybean has been shown to be sensitive to weed interference, which is of great importance during the development of the crop. Weeds can compete for environmental resources and release allelopathic substances (Ariuan et al. 2016). Weed monitoring and weed control management are influential factors in field crop production, especially in relation with yield formation (Kassai et al. 2007; Kende et al. 2020).

Soybean crop is frequently exposed to game damages. Some authors have stated however, that from among game damages rabbit bite causes minor losses only (De Calesta and Schwendeman, 1978). MacGowan et al (2007) found rabbits very effective in causing yield depression especially at the edges of crop fields. The magnitude of crop yield losses could be highly correlated with the rabbit population in a Hungarian experiment.

## Materials and Methods

### *Open-field experiment*

A field experiment was carried out at the experimental site of the MATE Department of Agronomy in Gödöllő, Hungary (47°46'N, 19°21'E, 242 m above sea level), on a sandy loam, brown forest soil (Chromic Luvisol) during the 2020 growing season. The experimental site is located in a hilly area with a close to average climatic zone of the country. The 2020 year was exposed to slightly higher precipitation. The annual

average precipitation of Hungary was 615 mm in 2020, while the respective value of Gödöllő was 694 mm. 12.8% higher than that. The actual crop years temperature means did not differ from the average.

A soybean variety used in the trial was ES Gladiator. It was planted with a scheduled plant density of 540 000 viable germs on a hectare. The experimental design was a 2 × 3 factorial arranged in a split plot design with four replications. In this experimental design, nitrogen fertilizer was assigned to the main plot and weed canopy to the sub-plot (Table 1.)

The experimental plot was cleared, ploughed, rotor-tilled and seedbed was prepared before planting. The basic fertilizer treatments were applied to the experimental field in accordance with the usual practices (Birkás et al 2004) following soil analysis data. A preemergent weed control was used to eliminate weeds by Targa Super EC. Soybean seeds were planted at a depth of 3 cm. After eleven weeks of planting, the plants were supplied with nutrition according to the treatments which were no nutrient supply (control) and supplied with 200 kg N/ha. Weeds were controlled every two weeks according to the weed canopy treatments which were weedy, hand weeded and mechanically weeded. Plant development, plant density, rabbit bite damages were monitored a recorded with phenological observations. The plants were then harvested manually. Planting and harvest dates were respectively



Table 2: The decline in plant density during the vegetation period, % by observation date

TRT	22.07.20	12.08.20	24.08.20	04.09.20	10.09.20	17.09.20	07.10.20
N1W1	100	100	100	96	96	93	91
N1W2	100	100	100	100	100	100	93
N1W3	100	100	100	95	95	95	79*
N2W1	100	100	100	100	100	100	100
N2W2	100	100	100	96	93	93	93
N2W3	100	100	100	95	95	95	87*
Mean	100	100	100	97	96	96	91

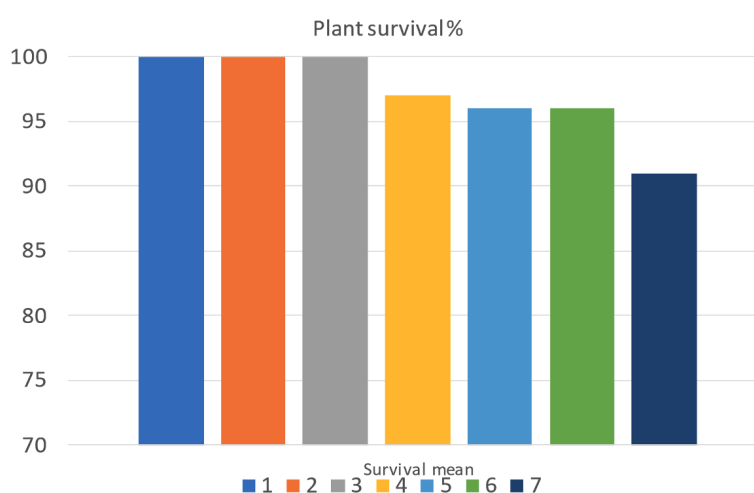


Figure 1: Plant survival average of the trial, %

on the 25<sup>th</sup> May and 7<sup>th</sup> October.

At harvest, all the plants in a sampling area of 1 row meter in each plot were harvested to calculate grain yield. Pods from harvested plants were oven-dried immediately at temperature of 50 °C for two days for grain yield determination. The dried pods then were hand-threshed and the grains were weighed to calculate grain yield per plot. All seed samples were analysed at the laboratory of the MATE Institute of Agronomy.

Statistically, a one-way between treatments ANOVA was conducted to compare the effect of the different nutrition supply and weed canopy. ANOVA was performed at  $p = 0.05$  level of significance to determine

whether the treatments were different. Post hoc comparisons using the least significant difference (LSD) test was made at  $p < 0.05$ . For the statistical evaluation of our results, we used the Explore and ANOVA modules of the IBM SPSS V.23 software.

## Results and discussion

The experimental plots were planted with a scheduled average of 7 viable seeds/row m. The first plant number count has recorded 6.375 plants/plot in average. This plant density was gradually reduced to 5.801 plants/plot, mainly due to rabbit bite damages. Altogether the plant survival was 91%

Table 3: Pod number count by time and by experimental treatments

TRT	24.08.20	04.09.20	10.09.20	17.09.20	07.10.20
N1W1	33	36	36	39	39
N1W2	47	49	51	57	57
N1W3	31	31	28	30	30
N2W1	39	40	41	40	40
N2W2	51	56	58	59	59
N2W3	32	36	40	43	47

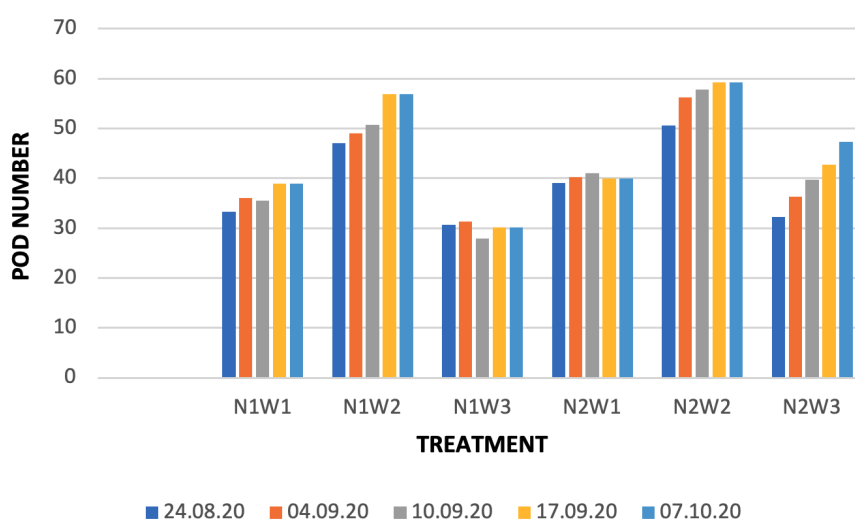


Figure 2: The increment of pod numbers by treatments

Table 4: 1000 grain weight, g

TRT	1000 grain weight (g)	
	Fresh	Dry
N1W1	144	112
N1W2	135	103
N1W3	128	98
N2W1	156	120
N2W2	160	123
N2W3	153	106

in average (Table 2).

The survival of plants was the best in the cases of control and hand weeded plots for both nutritional treatments. The decline started by the end of August and the first

rabbit damages have been recorded from September. From that date the survival gradually decreased until harvest. Significant differences were found only in the case of mechanically weeded applications and by the

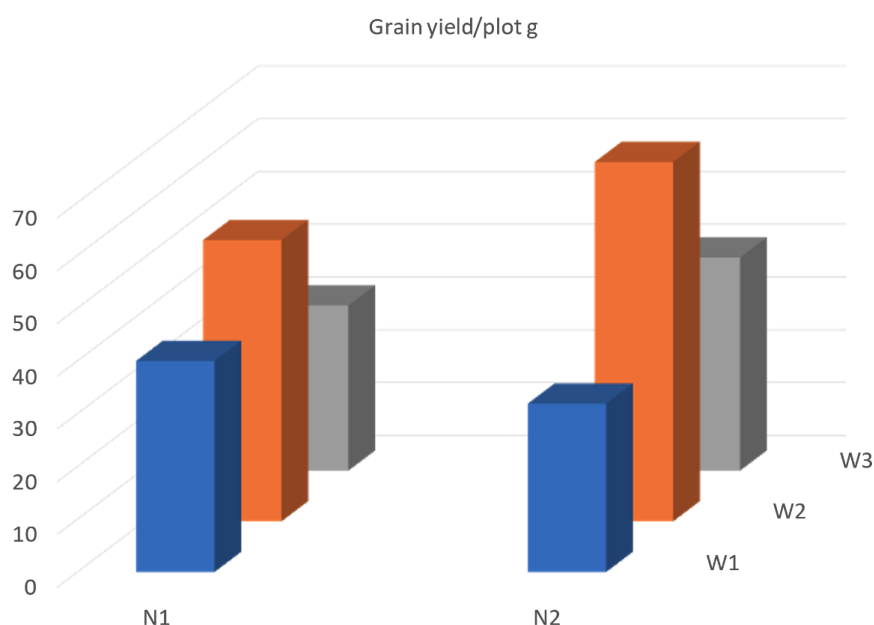


Figure 3: Total grain yields, g

last observation date (Fig 1).

Pod number performance of plots were rather diverse in accordance with the vegetation period and the treatments applied (Table 3). In general, it can be stated that the highest pod numbers were developed by plants of hand weeded plots. Nitrogen applications did not have a direct effect on pod number. Number of pods increased with time in most applications, however this consequent increment within treatment was not significant as it is demonstrated by Fig 2.

The harvested grain yield has shown detectable differences between applications. There were no significant differences between the yields harvested from N application plots, however significant differences were recorded due to weed control applications. Grain was less influenced by the 1000 grain weight of the yield samples (Table 4).

The total grain yields are presented in Fig 3. There was no statistically significant difference between nutrition groups according to one-way ANOVA at the  $p < 0.05$  level. However, there was a statistically significant difference between weed canopy groups for

grain yield. Hand weeded versions had an almost twofold yield improving effect in the case of high N applications, and some 1.5 improvement in the case of no N treatments.

## Conclusion

Agri-environmental impacts on grain yield of ES Gladiator soybean variety have been studied in a replicated field trial at the Gödöllő experimental field, Hungary. N application and various means of weed control was studied, and samples of grain yield were evaluated in accordance with the treatments. The results obtained suggest, that N topdressing had positive, but no significant effect on grain yield, while the means of weed control resulted in an almost twofold yield improvement compared to the control. Rabbit bite damages were recorded during yield formation phenophases. The extent of game damage was consequent but not significant regarding crop yield.

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## Sustainable tourism activities in Green Star hotels: A research in Europe

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**Abstract:** The unconscious use of the resources on earth caused great problems with the economic, social and cultural developments experienced after the industrial revolution. The tourism sector is a sector that is integrated with environmental resources, develops and gains importance depending on these resources. In terms of efficient use of limited resources within the framework of tourism sustainability dimensions of activities in hotel enterprises: to establish a reduction, reuse and recycling system. In this way, prevention of unnecessary use, more efficient use and reuse shows that green star hotels are one of the important determinants of sustainable tourism. Sustainable tourism activities in the hospitality sector are growing worldwide. The purpose of this study is to reveal the activities of green star hotels within the scope of sustainable tourism. In this context, it has been achieved by semi-structured interview technique with managers working in green star hotels in Europe. Energy consumption, water consumption waste consumption, and CO<sub>2</sub> management sustainable activities are carried out in green star hotels as environmental dimensions were evaluated.

**Keywords:** Sustainability, Sustainable Tourism, Sustainable Development, Green Star

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### Introduction

The concept of sustainability is based on the principle of protecting economic development, environmental resources and values and transferring them to future generations. When the concept of sustainability is applied to tourism, it can be expressed as the regulation of the use of tourism resources in a way that will enable future generations to use them without being consumed, polluted and destroyed (Kozak, 2014). Middleton and Hawkins (1998) defined sustainability as "a state of balance in which there is harmony between the activities of the human population and their natural, social and cultural environment". According to Coccossis (1996), sustainability is "a set of approaches aimed at preventing economic, social and environmental negativities arising from the effects of human activities on environmental resources and emerging in the long term".

"Friendly environment" or "green" oriented

plans and policies that have become increasingly important for large and small businesses after the 1990 (Revilla et al. 2001). It has quickly become the subject of the applications of the accommodation sector, which is one of the key elements of the tourism industry. The concept of green hotels, which is used to describe the hotels established by investing from scratch with green strategies or through modernization, defines the hotels that produce quality service by using efficient energy, water, waste management and natural resources in a respectful way to nature (Cooper, 1998). According to the definition made by the Green Hotels Association, green hotels are "environmentally friendly businesses that make the necessary practices to save water and energy and reduce waste and help protect the world we have" (Lee et al. 2010). In order to be called as green hotels through green strategies, accommodation businesses must pass certain controls and show that they are sufficient in terms of

air quality, energy, water and waste management (Middleton and Hawkins, 1998). Although the high number of these certificates makes it difficult to define, green certificates can be defined as "effective instruments that improve the environmental quality of the tourism region while minimizing the damage caused by the products, production methods, services and processes produced by the tourism industry" (Kozak, 2014).

19th century, the rapid industrialization efforts that emerged as a result of the developments in the economic, social, cultural, environmental and technological fields with the Industrial Revolution caused irreparable damages on natural resources and the environment (Mebratu, 1998). In the 1970s, various meetings were organized under the leadership of international organizations such as the United Nations and the World Bank in order to prevent these damages to the environment and natural resources, and as a result of these meetings, the concepts of "Sustainability" and "Sustainable Development" emerged (Cunningham, 2004). Sustainability principles have been adapted within the tourism sector as in agriculture, industry and many other sectors, and the concepts and principles of "Sustainable Tourism" have been developed under the leadership of the World Tourism Organization and the United Nations (Despotakis, 1987).

Moreno, Lorento, and Jimenez (2004), in a study examining the environmental strategies adopted in the service sector and their effects on the performance of a company, found that the environmental strategies of Spanish hotels in their research findings on environmental protection activities are related to the higher performance level of the companies in the group with more advanced environmental strategies, but emphasized that it has nothing to do with performance. Kapiki (2012) in eco-friendly hotels, it is emphasized that green activities applied in hotels within the scope of sus-

tainable tourism provide cost benefits and increase customer satisfaction. At the same time, it was emphasized that the competitiveness of hotels with green management increased (Revila, et al. 2001). Amran et al. (2017), in the study of 115 hotels in Perlis, shows that hoteliers are starting to show a positive sign of adopting environmental practices such as the use of energy-efficient light bulbs, water-saving and recycling management programs. In addition Mungai and Irungu (2013), in research on green activities in 4 \* and 5 \* hotels in Mombasa, Kenya, it has revealed that the most water consumption was prevented from four general categories: energy management, waste management, water saving and green supply / recycling. Hays and Dosen (2014), in their research, indirect activities that save energy and water highlighted the importance of the proper recycling of waste. Not only do they reduce costs, they also build trust in green activities and thus add value to hotel services and brand. According to Sharma (2019) research on eco-friendly hotels in North India, green hotels recycle waste products and water, reduce the use of paper, ban the use of plastic and other toxic elements, customer intentions and customers such as the characteristics of products and services made in a sustainable way. it has been put on the ground that it directed to choose. In addition, Manaktola and Jauhari (2007) found that environmentally friendly practices are determinants of consumer preferences and choices as a result of their research. In addition, the green hotel where consumers stay; It has been concluded that participating in the environmental certification program, performing recycling practices and offering environmentally friendly products and services are effective in the customers' selection of green hotels.

In addition to the evaluation programs specific to sustainable hotel businesses such as internationally recognized Green Key (Denmark), Eco label (Worldwide), Green Leaf

Table 1: Energy management Activities

---

To close the curtains of empty rooms.
Staff are advised to turn off lights in not use rooms.
Installing LED bulbs to replace the halogen bulbs.
Hotels have solar panels.
Tesla Destination electric vehicle charging point.
Heating system is controlled automatically in the general areas of the hotels.
Lighting sensors are used in public areas.

---

(Thailand), Nordic Swan (Scandinavia), EU Flower (European Union), There is a Green Globe certification program, which is given to environmentally friendly accommodation facilities within the framework of social responsibility of hotels (Darnall, 2008; Honey and Rome, 2001). One of the most important and developing program which is established by The World Travel and Tourism Council (WTTC) The Green Globe in 1993 and is a worldwide leading certification. The Green Globe's worldwide network is recognized in 187 countries. Green Globe Certificate fully complies with ISO 17021 standards. The figures show that there are more than 800 certified businesses. The Green Globe certification program creates plans for energy, water, waste consumption as well as CO<sub>2</sub> management in hotels (<https://greenglobe.com>).

The aim of the research, which has been prepared based on all these issues, is to reveal the implementation activities in 5\* hotels with green globe certification in Europe within the scope of sustainable tourism. The protection of natural and cultural wealth plays an important role in ensuring sustainability in tourism. For this reason, it is necessary to continue sustainable development in tourism and sustainability studies. The research is important in terms of the activities of accommodation businesses, which are one of the most important stakeholders of the tourism sector, in terms of minimizing their damage to the environment and provid-

ing better service.

### **The Aim and Importance of the Research Method**

The aim of this research is to reveal the applications and environmental contributions of 5\* Green Globe hotels in Europe. Highlighting the activities related to the environment in Europe and the applications made in the field of tourism, as well as the applications of the Green Globe member hotels have been revealed.

As one of the sectors where environmental factors are important, the protection of natural, historical and cultural resources that constitute the input of the tourism sector, in other words, prevention of their destruction is of great importance for the existence, development and continuity of the sector. One of the most important stakeholders of the tourism sector, the damage they give to the environment due to the activities of accommodation businesses is to be minimized and to provide better service.

### **The Universe and Sample of the Research**

The number of sustainable hotels with Green Globe certification in Europe is 245. However, as the research will examine the practices of hotel businesses within the scope of

Table 2: Waste management Activities

Organic linen: all of our linen certified organic	Organic (refillable) amenities.
Proper disposal of used chemicals.	Non single use plastic.
Waste compactors, the waste registering in a hotel optimizer.	It use to waste compactors, the waste registering in a hotel optimizer.
Toiletries are biological and biodegradable.	Composting kitchen waste.

sustainable tourism, the sample of the study consists of 22 5\* hotels with Green Globe certification.

### Data Collection Process

In this research, as a data collection method, information about the applications in the hotels with the Green Globe Certification Program within the scope of sustainable tourism is collected from the websites of the hotels.

### Results

#### *Energy management*

Hotels are very committed to reducing energy consumption in all its facilities, in the past has implemented some measures to make this reduction (Table 1).

Hotels have just implemented a computer shutdown policy that operates where it is feasible. They have the policy to close the curtains of empty rooms, to regulate the temperature of the building, which leads to a reduction in the use of energy. In addition, Staff are advised to turn off lights in rooms that are not in use, such as their offices. Hotels also installing LED bulbs to replace the halogen bulbs that we have in the hotel.

#### *Waste Management*

An area in which the hotel feels very committed through the system of continuous improvement of waste management. Hotels use new recycling containers so that recycling is

easier according to the type of waste. To control waste management, all recycling is done in the back of hotels, besides, has begun to record the amount of waste it generates and what type they are to create long-term waste reduction plans (Table 2).

Hotels have also begun to use waste compactors. The compactors will not only help to reduce the amount of waste produced but also reduce greenhouse gas emissions, reducing the number of times per month that waste must be collected so that it will not only generate a positive impact for the environment but also that will also allow saving costs to the company, also, recently we have begun to register the waste in a hotel optimizer we will use this data to create recycling objectives of the departments.

Non-single-use plastic, all plastic items are substitute with biodegradable options; i.e.: slippers, straws. Organic (refillable) amenities, locally produced organic amenities available to guests without needing to use disposable bottles.

#### *Water Management*

This is an area where hotel guests can actively participate since they can communicate that they do not want their sheets and towels washed by placing the note on their bed and with their towels. Flow reducers and movement sensors have been installed in male urinals and toilets with double discharge in all hotel services to reduce the amount of water consumed by guests without lowering quality standards (Table 3).



Table 3: Energy management Activities

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On request sheets and towels change.
Flow reducers and movement sensors have been installed in toilets and all hotel services.
Rainwater is used to irrigate the garden and has a water drainage system.
Toilet flush is water conserving.

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Table 4: CO<sub>2</sub> management Activities

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Hotel register and evaluate their CO <sub>2</sub> emissions monthly .
Water plant uses advanced heat recovery technology.
Hotels use energy-efficient computers and other machines.

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*CO<sub>2</sub> Management*

Guests and visitors of the hotel also are informed of the best ways to move around the city sustainably. Hotels use energy-efficient computers and other machines, which are Energy Star certified or other energy efficiency certificates. Water plant uses advanced heat recovery technology to extract heat from cooled areas and then reuses it to create hot water. This technology delivers high output for low input, leading to a direct saving in CO<sub>2</sub> emissions and running costs. Hotels use energy-efficient computers and other machines, which are Energy Star certified or other energy efficiency certificates (Table 4).

However, education and training of staff in regards to waste separation and recycling take place on regular basis. It also takes part in socio-cultural activities in hotels. Hotels have a global perspective of its socio-cultural work and integration of other cultures, so it welcomes guests and workers from all over the world.

The training of its employees reflects this global perspective to ensure that all its workers have a better understanding of both the local and cultural culture of the guests and visitors who come to the hotel. Guests must be properly treated according to their cul-

ture, so the staff has been trained for better understanding the culture of each of their clients, thus increasing their final satisfaction with the company. From here hotels support the development of the local culture of the world, so they try to integrate it as much as possible in the establishment in the same way we support the intellectual property of the local culture. In the same way that local employees are trained to be able to serve the needs of their clients.

**Conclusion**

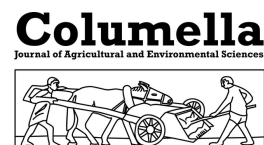
As a result of tourism activities, economic development and protection of environmental values, even increasing these values are the main objectives for sustainable tourism development. Making tourism sustainable depends on the elimination of some negativities and bold initiatives. In addition, " Water saving, increasing energy efficiency, reducing the consumption of environmentally hazardous substances and the amount of waste, encouraging the use of renewable energy resources, planning accommodation businesses environmentally sensitive starting from the investment stage, adapting the touristic facility to the environment, orga-

nizing and activities enhancing the environment, ecological architecture, It provides to raise awareness about environmental awareness, to provide training and to cooperate with relevant institutions and organizations. Although it was stated that the green star certification was high financially at first, it became the first choice of the enterprises

thanks to the financial and moral contributions it provided to the business. Hotel managements and personnel staff have completed the necessary trainings and continuity has been adopted as a principle, and with this, other gains have become an effective factor in the participation of the surrounding hotels in the green star certification program.

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[www.columella.hu](http://www.columella.hu)

## Reuse of wastewater from fish farm for irrigation in aerobic rice (*Oryza sativa* L.) cultivation

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**Abstract:** The utilization of wastewater in irrigated agriculture is becoming vital in those regions where access to freshwater is low. Wastewater in rice cultivation as an alternative water source can also play significant role in the future. This experiment was conducted in Szarvas, Hungary to evaluate the performance of a Hungarian rice variety ('Janka') irrigated with a saline effluent water from an intensive fish farm. Irrigation was carried out both in the form of direct use (I1) of the wastewater and with two additional treatments: I2 - gypsum supplementation, I3 - wastewater dilution with oxbow lake water and gypsum supplementation. Water from the local oxbow lake was set as irrigation control. During the experiment, the quality parameters, grain size and mineral composition (Na, K, Mg, Ca, and P) of rice seeds were measured. The study showed that the application of wastewater and treated wastewater decreased many of these parameters. I1 significantly reduced the thousand grain weight of paddy and cargo seeds. Meanwhile, I2 and I3 had a significant negative effect on the head rice percentage. Grain length, width and ratio were also decreased significantly compared to the control irrigation. Only the mineral content of the grains remained statistically unchanged. The current study showed that the selected rice variety generally reacted negatively to the wastewater and also to the treated wastewater.

**Keywords:** Rice; quality parameters; grain dimensions; mineral content; aquaculture effluent

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### Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops that is widely grown all over the world like wheat and maize (Maclean et al. 2013). The rice plant is commonly referred as the queen of cereals because of its mineral wealth (Anjum et al. 2007). Rice is rich in several important proteins and minerals (Verma 2011), however, mono diet can cause many health issues too (e.g. Vitamin A deficiency). Beside breeding approaches, production conditions (soil, irrigation water, weather, technology) have a significant effect on the nutrient uptake of rice plants (Gurusamy et al. 2007).

Rice goes through several production stages before reaching the consumers' table. After harvest, mechanical drying and cleaning is usually used before storage. To obtain cargo (or brown) rice, indigestible husk must be removed. During the final milling steps, bran from brown rice is polished and white rice is received (IRRI 2013). As these steps are implemented, rice undergoes not only changes in weight and shape, but also loss of minerals (Juliano 1993). Although brown rice has higher mineral content, white rice is much more popular in most countries because of the easier storage and cooking ability (Danquah and Egyir 2014).

Like other crops, rice also depends on the proper supply of nutrients to ensure a high and healthy harvest (Sasaki et al. 2016; Che et al. 2018). The absorption of nutrients by the rice plant is a complex process that involves the relationship between soil, water and air. The minerals, taken from the roots of the rice, are transmitted to the above-ground parts, circulate during the growing season and provide plant growth. The use of different treatments plays an essential role in improving the yield-attributing parameters of rice (Pati et al. 2016). For instance, the application of potassium in the manner proposed by Hu and Wang (2004) can lead to an increase in the nutrient uptake and nutrient use efficiency of rice. Many factors can affect the mineral content of plants, one of that is the amount of available water (Martínez-Ballesta et al. 2010).

Plants need water to maintain normal development and enzymatic activities. Moreover, water dissolves nutrients in the soil and ensures their availability for absorption by plants (Fipps 2003). Nowadays, water is a main limiting factor for agriculture in many regions and one of the biggest challenges for farmers to maintain appropriate water supply. Because of this, alternative water resources are becoming more and more important to meet the water demand for irrigation (Birol et al. 2010). Wastewater or effluent water irrigation is one of the leading alternatives among these methods (Zhang et al. 2010). Among other parameters, quality and quantity of different irrigation water types from conventional and alternative sources have a significant role on the productive parameters and chemical composition of crop plants (Eid and Hoballah 2014).

Water scarcity, competition for water resources, high cost of water and fertilisers have made the wastewater application attractive. Wastewater, in addition to the elements that plants need, can also contain heavy metals, pathogens that can damage

them. From this point of view, Soothar et al. (2018) did not suggest the direct application of wastewater in rice cultivation. Mukherjee et al. (2013) noted that the presence of lead and mercury in wastewater affected the rice plant and caused economic damage to farmers. On the other hand, during the use of wastewater, farmers have direct contact with them, which raises concerns about health issues (Pham and Watanabe 2017). However, most researchers believe that positive results can be obtained by choosing a tolerant rice variety and proper wastewater treatment. The use of reclaimed wastewater, especially in arid and semi-arid zones, where the salinity of fresh water is higher, gives more effective results (Kaboosi and Esmailnezhad 2018). For example, with suitable dilution in accordance with special standards, rice performance immediately increases from the initial stage (Kang et al. 2004; Dash 2012; Gasama et al. 2015; Akhtar et al. 2018). According to some studies, reclaimed wastewater does not have a side effect on human health (Papadopoulos et al. 2009; Jang et al. 2013). Moreover, in some cases, during irrigation with wastewater, rice yield may be higher than with conventional sources of water (Yoon et al. 2001; Kang et al. 2007).

The current study involves investigation of the effect of fish farm effluent water on aerobic rice as a follow-up experiment of previously published studies (Ibadzade et al. 2020). The main focus was on plant performance by measuring quality parameters, grain dimensions and grain mineral composition.

## Materials and Methods

### *Experimental area and design*

The research area is located in the Lysimeter Station (46°51'48"N, 20°31'39"E) of the Hungarian University of Agriculture and Life Sciences, Institute of Environmental Sciences, Research Centre of Irrigation and

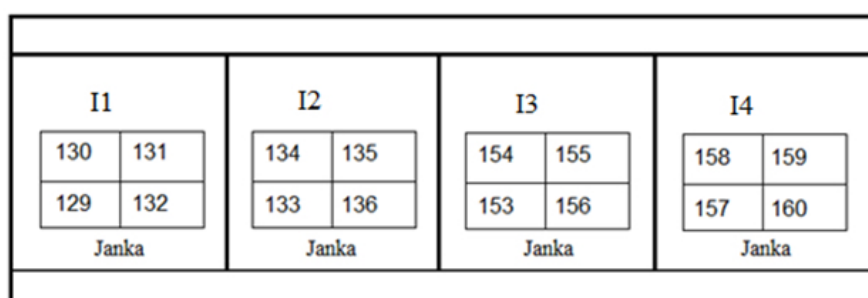


Figure 1: Schematic representation of experimental design. I1: wastewater, I2: gypsum supplemented wastewater, I3: wastewater diluted with surface water and supplemented with gypsum, I4: oxbow lake water (control). Number in cells: lysimeter ID number. Janka: Hungarian rice.

Table 1: The chemical characteristics of irrigation water used in the experiment. I1: wastewater, I2: gypsum supplemented wastewater, I3: wastewater diluted with oxbow lake water and supplemented with gypsum, I4: oxbow lake water (control). M: mean. SD: standard deviation.

Parameters	I1 M ± SD	I2 M ± SD	I3 M ± SD	I4 M ± SD
pH	7.77±0.12	7.71±0.12	7.70±0.15	7.55±0.04
EC (µS cm <sup>-1</sup> )	1180.00±125.30	1905.00±125.30	1033.75±208.33	371.86±20.14
TN (mg L <sup>-1</sup> )	26.30±3.04	28.55±3.04	13.10±2.53	1.19±0.09
TP (mg L <sup>-1</sup> )	2.18±0.13	2.67±0.13	1.53±0.68	0.15±0.04
SO <sub>4</sub> <sup>2-</sup> (mg L <sup>-1</sup> )	32.65±2.19	448.75±2.19	164.18±103.00	34.58±3.20
Ca (mg L <sup>-1</sup> )	23.23±1.35	187.50±1.35	90.83±31.11	39.04±0.73
Mg (mg L <sup>-1</sup> )	10.08±0.86	11.02±0.86	10.69±1.05	9.80±0.56
Na (mg L <sup>-1</sup> )	249.00±47.16	266.75±47.16	131.25±12.84	28.90±4.01
K (mg L <sup>-1</sup> )	6.08±0.75	6.61±0.75	5.43±0.35	3.71±0.70

Water Management (MATE IES ÖVKI) in Szarvas, Hungary. The experiment was carried out in 16 non-weighing lysimeters with a volume of 1 m<sup>3</sup> in 4 repetitions (Figure 1). The soil used in these gravitational lysimeters is classified as Vertisol (expansive clay). Untreated wastewater from a local intensive African catfish farm was used and directly collected from the outflow of the fish rearing tanks. The flow-through system of the fish tanks is supported by a geothermal well from a confined aquifer. Because of the geothermal origin, the effluent water also carrying high content of total salinity including high

amount of sodium (Table 1).

The use of wastewater for irrigation was performed as: direct use of wastewater – I1; gypsum supplemented wastewater – I2; wastewater diluted with oxbow lake water and supplemented with gypsum – I3. For control treatment, the water of the nearby oxbow lake (I4) was applied (Szarvas-Békésszentandrás Holt-Körös, Szarvas).

#### *Crop management, irrigation and weather*

In our experiment, a Hungarian rice variety 'Janka' (temperate japonica) was used. The cultivation of the plants was carried out un-

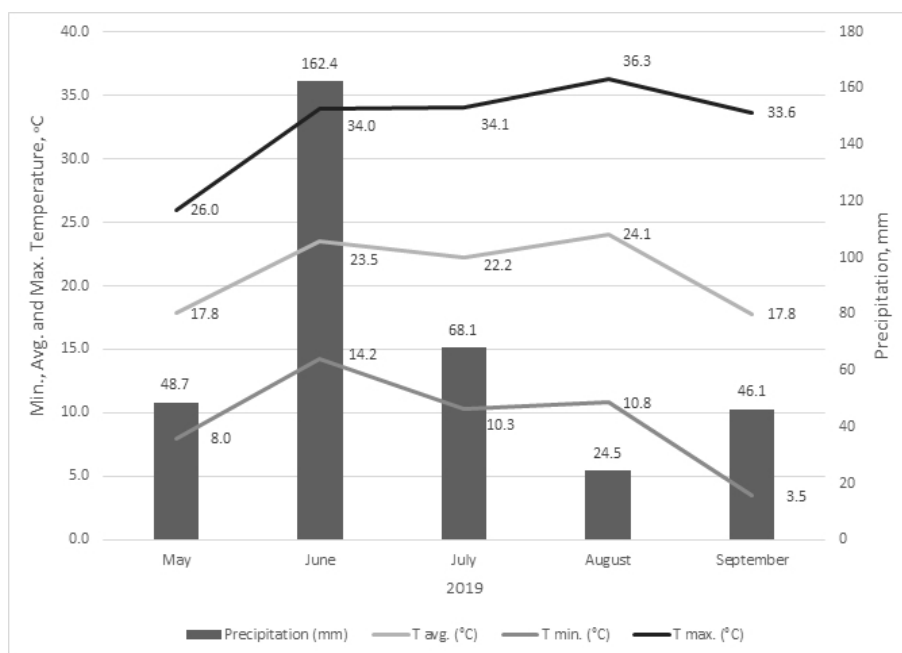


Figure 2: Monthly precipitation; Average, Minimum and Maximum temperature.

der aerobic conditions in large non-weighing lysimeters.

Rice seeds were sown manually on the 22<sup>nd</sup> of May, 2019 and 0.5 kg of fertiliser ( $\text{NH}_4\text{NO}_3 + \text{CaMg}(\text{CO}_3)_2$ ) was applied ( $84.4 \text{ kg Nha}^{-1}$ ) only once during the growing season. Irrigation of plants was carried out in all the treatments with a commercially available micro-sprinkler irrigation system. At the beginning of the experiment, 20 mm of river water was applied twice on all of the lysimeters to achieve uniform emergence. After that, irrigation schedule was planned as a weekly dose of 20 mm, which was modified according to the meteorological conditions. During the growing season (May-August), the amount of irrigation water for all the treatments was as total 160 mm. Dates of irrigations were 14<sup>th</sup> of June, 2<sup>nd</sup>, 4<sup>th</sup>, 12<sup>th</sup>, 18<sup>th</sup>, and 26<sup>th</sup> of July, 12<sup>th</sup> and 22<sup>nd</sup> of August, respectively. The crop was harvested manually on the 24<sup>th</sup> September, 2019.

All primary meteorological data for the experiment were received from an automatic weather station (Agromet-Solar, Boreas Ltd.,

Hungary) which were set next to the experimental site (Figure 2).

### Measurements

#### Determination of 1000 Grain Weight (TGW)

A sample of 100 seeds was weighed to measure paddy seeds, then the husks were removed with a Satake THU Laboratory Husker equipment and the cargo rice was weighed on analytical balance (Sartorius BP221S), and then the result was multiplied by ten. The test was repeated four times for the whole sample, and the average thousand grain weight of paddy (TGWp) and cargo (TGWc) rice was calculated.

#### Determination of Head Rice Percentage (HRP)

A husk layer of 100 g of seeds from each sample was removed and weighed. Later, brown grain was polished with Satake TM05 Test Mill laboratory equipment from these samples, and the result was weighed. The whole polished rice (white grain) was separated and weighed in four repetitions.

#### Grain Dimensions (GD)

In order to measure grain size and shape, 100

seeds from every sample were scanned using a flatbed scanner (HP ScanJet Pro 3500 f1) and Readiris Pro 14.1 software into “tiff” format. The scanned pictures were transferred to “jpg” format and were analysed using SmartGrain 1.2 software. These steps were repeated four times for the samples and the mean length (mm), width (mm) and L/W ratio were calculated.

#### *Mineral composition (MC)*

Based on MSZ EN ISO 11885:2000 international and Hungarian standards, in this experiment we analysed the mineral composition in rice grains, i.e. the content of Na, K, Mg, Ca and P. Na, K, Mg and Ca were measured with a Thermo Scientific Solaar M6 Atomic Absorption Spectrophotometer, and P was determined using Thermo Scientific ICAP 6000 Inductively Coupled Plasma Atomic Emissions at the MATE ÖVKI Laboratory for Environmental Analytics (Szarvas, Hungary).

#### *Statistical analysis*

The data were analysed by using of IBM SPSS software (version 22). To compare the effect of every irrigation treatments, data were tested through one-way analysis of variance (ANOVA). Differences between means were compared ( $p < 0.05$ ) by Tukey's test.

## **Results and discussion**

#### *Quality parameters*

The results of thousand grain weight tests of paddy (TGWp) and cargo (TGWc) seed of the Janka rice variety are illustrated in Table 2. There was a significant effect of treatments on TGWp at the  $p < 0.05$  level [ $F(3, 12) = 14.6, p = 0.001$ ]. Post hoc comparisons using the Tukey HSD test showed that the mean TGWp in I1 irrigation ( $24.59 \pm 0.56$ ) was significantly lower than the I4 ( $26.45 \pm 0.56, p < 0.001$ ), I2 ( $26.08 \pm 0.29, p < 0.01$ ) and I3 ( $25.86 \pm 0.46, p <$

$0.01$ ) irrigations. But there was no significant difference between I2 and I3 irrigations ( $p > 0.05$ ) on TGWp.

An analogous result was also observed in TGWc [ $F(3, 12) = 7.66, p = 0.004$ ] (Table 2). While there was no statistically significant difference between I2, I3 and I4 ( $p > 0.05$ ); but between I1 ( $20.05 \pm 0.43$ ) and I2 ( $21.16 \pm 0.37, p < 0.05$ ), I3 ( $20.93 \pm 0.42, p < 0.05$ ), I4 ( $26.45 \pm 0.33, p < 0.01$ ) the difference was statistically significant.

During the milling stage (Table 3), the effect of irrigation with different water sources was not statistically significant for the percentage of brown and white grain of ‘Janka’ ( $p > 0.05$ ). However, significant differences in HRP (Table 3) were determined [ $F(3, 16) = 10.69, p = 0.001$ ]. Irrigation with I2 and I3 reduced the HRP, and there was a statistically significant difference between the control ( $56.08 \pm 3.26$ ) and I2 ( $42.64 \pm 2.54, p < 0.001$ ), I3 ( $47.12 \pm 5.47, p < 0.05$ ). Only between I1 and I4 was found a non-significant difference ( $p > 0.05$ ).

Typically, rice plants respond positively to the environment with an optimal level of nutrition (Jahan et al. 2017). For instance, different levels of P and N fertilisers can increase thousand grain weights at an important degree (Hasanuzzaman et al. 2012; Yosef Tabar 2012). However, regardless of the rich mineral composition of wastewater, Kaboosi and Esmailnezhad (2018) in their experiment did not notice significant changes in the weight of thousand grains. Duy Pham et al. (2019) also identified this trend under continuous irrigation with treated wastewater. In irrigation water with a high percentage of sodium, the yield attributes of rice may drop drastically (Rahman et al. 2017). In the current experiment, the TGW decrease was more evident with the application of I1 irrigation. Basically, a decrease in these values can also be explained by the presence of sodium in wastewater. Similar result was noted by Abdullah et al. (2002) as well.

Table 2: TGW of paddy and cargo seeds of rice developed with different irrigation water. I1: wastewater, I2: gypsum supplemented wastewater, I3: wastewater diluted with oxbow lake water and supplemented with gypsum, I4: oxbow lake water (control). M: mean. SD: standard deviation. Values followed by the same letter do not differ significantly from each other at 0.05 level according Tukey's honestly significant difference (HSD) post hoc test.

Irrigation treatments		TGWp (g)	TGWc (g)
I1	M	24.59 <sup>a</sup>	20.05 <sup>a</sup>
	SD	0.56	0.43
I2	M	26.08 <sup>b</sup>	21.16 <sup>b</sup>
	SD	0.29	0.37
I3	M	25.86 <sup>b</sup>	20.93 <sup>b</sup>
	SD	0.46	0.42
I4	M	26.45 <sup>b</sup>	21.3 <sup>b</sup>
	SD	0.33	0.40

Table 3: Milling fraction of rice grain developed with different irrigation water. I1: wastewater, I2: gypsum supplemented wastewater, I3: wastewater diluted with oxbow lake water and supplemented with gypsum, I4: oxbow lake water (control). M: mean. SD: standard deviation. Values followed by the same letter do not differ significantly from each other at 0.05 level according Tukey's honestly significant difference (HSD) post hoc test.

Irrigation treatments		Brown grain (%)	White grain (%)	Head rice (%)
I1	M	77.92 <sup>a</sup>	67.44 <sup>a</sup>	52.08 <sup>bc</sup>
	SD	1.04	1.51	4.13
I2	M	77.44 <sup>a</sup>	67.20 <sup>a</sup>	42.64 <sup>a</sup>
	SD	0.54	0.28	2.54
I3	M	78.64 <sup>a</sup>	68.96 <sup>a</sup>	47.12 <sup>ab</sup>
	SD	0.67	1.08	5.47
I4	M	78.64 <sup>a</sup>	68.80 <sup>a</sup>	56.08 <sup>c</sup>
	SD	1.35	1.67	3.26

Moreover, according to Chunthaburee et al. (2015), salt-sensitive rice varieties are more likely to have this type of low result.

On the other hand, during I2 and I3 irrigation the HRP significantly decreased. Although the effect of treatments did not manifest itself in the TGW, it caused the loss of the HRP. Stress factors caused by sodium always led to negative changes in yield param-

eters (Alam et al. 2004), which in our case, despite an attempt to reduce the influence of sodium in wastewater, encountered a decrease in HRP.

#### *Grain dimensions*

Statistical analysis revealed that the treatments have resulted considerable changes in grain dimensions. There was a significant ef-



Table 4: Milling fraction of rice grain developed with different irrigation water. I1: wastewater, I2: gypsum supplemented wastewater, I3: wastewater diluted with oxbow lake water and supplemented with gypsum, I4: oxbow lake water (control). M: mean. SD: standard deviation. Values followed by the same letter do not differ significantly from each other at 0.05 level according Tukey's honestly significant difference (HSD) post hoc test.

Irrigation treatments		Length (mm)	Width (mm)	L/W Ratio (mm)
I1	M	9.49 <sup>a</sup>	2.93 <sup>ab</sup>	3.23 <sup>a</sup>
	SD	0.49	0.27	0.31
I2	M	9.68 <sup>b</sup>	2.89 <sup>a</sup>	3.29 <sup>a</sup>
	SD	0.53	0.25	0.27
I3	M	9.64 <sup>b</sup>	2.94 <sup>bc</sup>	3.24 <sup>a</sup>
	SD	0.48	0.22	0.29
I4	M	9.78 <sup>c</sup>	2.99 <sup>c</sup>	3.38 <sup>b</sup>
	SD	0.46	0.27	0.30

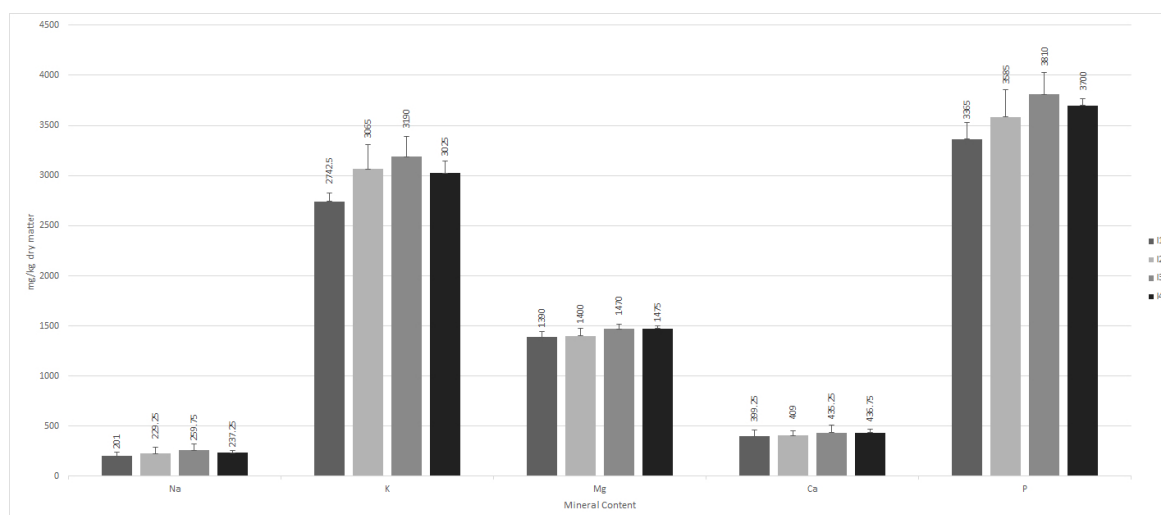


Figure 3: The mineral content in Janka rice grains developed with different irrigation water. I1: wastewater, I2: gypsum supplemented wastewater, I3: wastewater diluted with surface water and supplemented with gypsum, I4: oxbow lake water (control).

fect of treatments (Table 4) on grain length of Janka rice variety [ $F(3, 1578) = 24.01, p = 0.0001$ ]. Post hoc test indicated that the grain length was statistically lower after the irrigation with I1 ( $9.49 \pm 0.49, p < 0.001$ ), I2 ( $9.68 \pm 0.53, p < 0.05$ ) and I3 ( $9.64 \pm 0.48, p < 0.001$ ). The average length of grain after the I1 irrigation was statistically lower

even after I2 ( $p < 0.001$ ) and I3 ( $p < 0.001$ ) irrigations.

Grain width of rice (Table 4) was also affected by irrigation [ $F(3, 1586) = 8.38, p = 0.0001$ ]. The average grain width after I1 ( $2.93 \pm 0.27, p < 0.05$ ) and I2 ( $2.89 \pm 0.25, p < 0.001$ ) irrigations was significantly lower compared to the control irrigation

( $2.99 \pm 0.27$ ). The effect of I3 irrigation on grain width was non-significant ( $p = 0.051$ ). Irrigation with I1, I2, and I3 significantly reduced the average L/W ratio of Janka rice grains [ $F(3, 1561) = 21.27, p = 0.0001$ ]. The average L/W ratio of grains was  $3.23 \pm 0.31, 3.29 \pm 0.27, 3.24 \pm 0.29$  and  $3.38 \pm 0.30$  for I1, I2, I3 and I4 irrigation, respectively (Table 4).

As with drought conditions (Haider et al. 2015), salinity protection is also important for seed formation. Especially in the flowering stage, it is more necessary to avoid these undesirable factors (Yang et al. 2019). Altogether, in our experiment rice grain dimensions were negatively affected regardless of the irrigation treatments. Apparently, along with other parameters due to a stressful environment, the size of paddy seeds also changed. As Fabre et al. (2004) previously reported, the decrease in grain size is associated with stress conditions caused by salinity. According to Rao et al. (2013) while this varies depending on the level of stress tolerance, as the stress level increases, a decrease in grain dimensions is inevitable.

#### *Mineral Composition*

Among the studied parameters, the mineral composition of grains was the least variable during irrigation treatments (Figure 3). Although some changes were recorded in the Na, K, Mg, Ca and P content of grains, but this was not reflected in the statistical analysis. The effect of irrigation treatments as determined by one-way ANOVA was statistically similar to each other ( $p > 0.05$ ).

Nutrient distribution is a complex function that encompasses all plant cells (Wang et al. 2011). Usually, mineral accumulation can be impaired in salty environments (Huang et al. 2017). According to some studies, the sodium content increases considerably in different parts of the plant (Cha-um et al. 2007; García Morales et al. 2012). However, in this

experiment, we did not see any change in the mineral composition of the Janka rice grains after the treatments. Despite their rich nutrient content and high Na, the treatments could not influence the mineral composition of the grains, and the studied rice plant could maintain the grain mineral composition even in a moderate saline environment.

#### **Conclusions**

The current study showed that the selected rice variety generally reacted negatively to the wastewater and also to the treated wastewater. Taking into account the individual parameters of 'Janka', it was found that the thousand grain weight of paddy and cargo seeds decreased significantly after the I1, and the head rice percentage after the I2 and I3 irrigations, respectively. Moreover, all treatments have significantly reduced grain dimensions compared to the control. Although, the sodium in the wastewater played a decisive role in the development of plants and seeds. However, the wastewater treatments were higher in mineral concentration; they did not affect significantly the mineral composition of the grains. In this regard, the improvement of wastewater treatments and the cultivation of more salt tolerant rice varieties could promote reutilization and bioremediation approaches of wastewater irrigation in the coming years.

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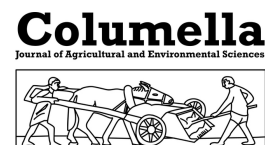
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[www.columella.hu](http://www.columella.hu)

## Yield components of soybean cover crop regard to seed pre-treatment with bacteria and mycorrhiza

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**Abstract:** The aim of this study was to describe the importance of bacterization of soybean seeds and the use of preparations of mycorrhizal fungi in the sowing of soybeans on the family farm "Alduk" in 2020. Post-sowing of soybeans was done on 26<sup>th</sup> June 2020 as cover crop. Two very early varieties (00 maturity group): *Korana* (Agricultural Institute Osijek) and *Merkur* (NS seeds, Serbia) were used. Before sowing, the seeds were bacterized (Nitrobacterin – Faculty of Agrobiotechnical Sciences Osijek) or the addition of preparations of mycorrhizal fungi (VAM + *Azotobacter chroococcum* – Faculty of Agrobiotechnical Sciences Osijek). At harvest, yield components of soybeans in 2020 were determined. To determine the yield components from each treatment, 20 plants were selected and analysed separately. A total of 120 individual plants were analysed, and the following were determined: plant height (cm) and height to the first pod, number of fertile levels per plant from the central stem and per plant, number of pods per plant and seed mass of one plant (g), 1000 grain mass and at final, seed yield (t/ha). The height of the plants up to the first fertile pod was on average 7 cm, and varied from 5 cm (Mercury variety with VAM + AC treatment), to 9 cm (*Korana* on NB treatment). The number of fertile levels per plant averaged 11 on the main, central stem, while the total number of fertile levels per plant was 16. The number of pods per plant in this study averaged 42, with the seed weight of one plant being 10.48 g per plant. The highest mass of seeds per plant (g) had the *Korana* variety on the control treatment (14.95 g per plant). The *Korana* variety also had the lowest seed mass per plant (7.07 g per plant) with the application of Nitrobacterin. According to the results, *Korana* variety had the highest yield on the control treatment (1.19 t/ha), followed by the treatment with VAM + *Azotobacter chroococcum* (1.04 t/ha) and the lowest with the application of Nitrobacterin (0.84 t/ha). *Merkur* variety had the lowest soybean yield on the control treatment (0.69 t/ha), while with Nitrobacterin and VAM + *Azotobacter chroococcum* soybean yield increased by about 19% with Nitrobacterin and about 27% with VAM + *Azotobacter chroococcum*.

**Keywords:** Cover crop, soybean, bacteria, mycorrhizae, yield components

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### Introduction

One of the biggest advantages of legumes is the symbiosis with bacteria that fix nitrogen from the atmosphere to the soil, which results in less nitrogen-fed mineral form, and over time reduces the cost of agricultural production. Legumes are a great pre-crop for small grains because they leave a lot of nitrogen in the soil that the next crop can use (Tucak et al., 2019). The most important representatives of legumes are soybeans, beans, peas and beans. Due to their nutri-

tional value, chemical composition and large amounts of protein grain and legume fruit are often used in human nutrition (Matoša Kočar et al., 2020).

Soybean, *Glycine max* (L.) Merrill, is a legume plant native to East Asia, where it has followed exceptional nutritional value for centuries as a food and medicine. It is grown from 20° to 60° north latitude. The agrotechnical significance of soybeans is in its symbiotic relationship with the nitrous nodule bacteria *Bradyrhizobium japonicum*, which through a natural process fixes inor-

ganic nitrogen ( $N_2$ ) from the air and converts it into an ammoniacal form ( $NH_4^+$ ) that approaches plants in exchange for carbohydrates (Lupwayi et al., 2000; Tokgöz et al., 2020). In this way, the need for mineral nutrition with nitrogen is reduced and enriched for the next crop in the crop rotation, which together significantly reduces production costs, and thus increases profits over time. Since biologically bound nitrogen is not leached from the soil, there is no leaching of nitrate into groundwater and eutrophication (Vratarić and Sudarić, 2000).

Soybeans first appeared in Croatia in 1876 in the area around Dubrovnik and in the north of the country, and were brought by the Austrian biochemist Friedrich Haberlandt (Vratarić and Sudarić, 2008). Until 1981, soybean production was at the level of the whole of Croatia only 3714 ha, which significantly depended on the year and the price on the world market. From 1987 can be considered the initial year of more stable soybean production in the country. In the period until 1997, there was a stabilization of soybean areas on about 20,000 ha, and the largest soybean areas in Croatia are in Slavonia and Baranja. Yields have been increasing from year to year, so since 2000 we have recorded an increase in yields up to 3.1 t/ha compared to the period until 2000, when yields ranged up to 1.4 t/ha. The largest sown area under soybean since 2000 was recorded in 2015, when 88,867 hectares of soybean area were sown. The highest yield was recorded in 2016, when it was 3.1 t/ha. The smallest area under soybeans was sown in 2008 just over 35,700 hectares, and the lowest yield in the period from 2000 to 2017 was recorded in 2003 when it amounted to a small 1.7 t/ha (Central Bureau of Statistics, 2020). The average soybean seed yield in the period from 2015 to 2019 was 3.2 t/ha and the harvested area was more than 83,000 ha (Figure 1).

Nitrogen is one of the most important elements in plant nutrition in agricultural pro-

duction (Basal and Szabó, 2019; Kristó et al., 2020). Nitrogen fixation is one of the five, and at the same time the last phase of nitrogen circulation in nature. It is preceded by nitrogen assimilation, ammonification, nitrification and denitrification. We know two types of nitrogen fixation, namely abiotic and biotic fixation. Biotic fixation is still divided into non-symbiotic and symbiotic. Plants can only absorb it in nitrate and ammonia form. Nitrogen fixation binds atmospheric nitrogen with the help of nitrogen fixing bacteria which are then used by leguminous plants. Nodule bacteria are not the only nitrogen fixatives. Mycorrhizae fungi can also fix nitrogen (Brundrett, 2009). Pre-sowing seed bacterization is a recommended measure in the cultivation of all legumes. It is especially important for soils where soybeans have never been grown or where soybeans have not been grown for a long time. The introduction of nitrogen fixing bacteria into the soil improves its structure, increases the protein content of soybeans, and saves nitrogen fertilizers for the next crop (Deaker et al., 2004). Pre-sowing bacterization is a standard practice in the production of legumes because it provides optimal conditions for creating a symbiotic relationship between nodule bacteria and legumes, which ultimately leads to the adoption of a significant amount of atmospheric nitrogen per hectare per year. Bacterization is considered successful if there are 15-30 well-developed nodules on each soybean plant (Milaković et al., 2012).

Milić et al. (2004) state that the annual share of fixed nitrogen in the yield is high, which justifies the use of highly effective strains in microbiological preparations for inoculation of legume seeds, allows the replacement of nitrogen from mineral fertilizers with biological nitrogen, and has economic and environmental justification. Microbiological preparations for inoculation do not pollute the soil, reduce the use of mineral nitrogen fertiliz-



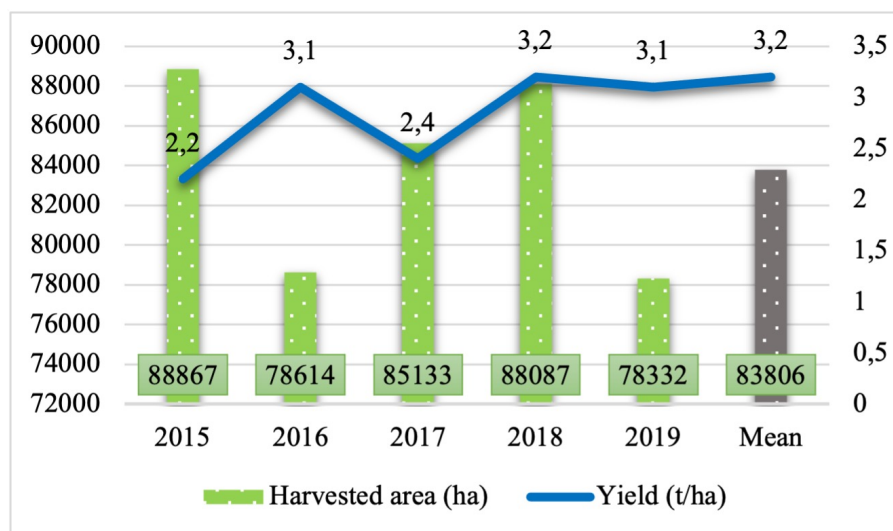


Figure 1: Soybean harvested area and seed yield in the Republic of Croatia (Central Bureau of Statistics, 2020).

ers, contribute to the production of ecologically healthy food, improve soil structure, increase the content of organic matter and positively affect the physical properties of the soil (Stevanović et al., 2017). Olsen (1982) recognizes the improvement of symbiotic nitrification in soybeans as part of an overall strategy to increase productivity and considers it necessary to pay attention to this in research work. Soybean and other legumes that have short root hairs, so association with mycorrhizal fungi is of paramount importance, resulting in better plant nutrition, such as, for example, the supply of phosphorus in the root (Fialho et al., 2016). Mycorrhizal symbiosis relationship implies that both participants benefit, in turn a particular plant supplies the fungi with photosynthesized food including sugar. In mycorrhiza, better nutrition (fungus promotes the absorption of water, carbon and nitrogen). Furthermore, fungi secrete enzymes that enable faster mineralization of soil organic matter and greater availability of nitrogen. In addition, fungi secrete acids that dissolve and absorb sparingly soluble minerals and transmit them from greater distances to the plant. The plant itself is more resistant and there is a greater

chance that the plant will survive in adverse climatic conditions because hyphae of the fungus act as a specific bioreservoir of water during drought.

The aim of the study was to describe the importance of bacterization of soybean seeds and the use of preparations of mycorrhizal fungi in the sowing of soybeans as a cover crop.

## Materials and Methods

### Field trail

An experiment of bacterization of soybean seeds and the use of mycorrhizal fungi in the sowing of soybeans was made at the family farm "Alduk" located in the Vukovar-Srijem County in Privlaka (Republic of Croatia). The family farm is managed by the owner of the same name, who took over the family farm founded in 2003 from his father Borislav. Currently, on about 210 ha, soybeans, wheat and corn are mostly sown. Soybeans have been sown for many years and have thus become an inevitable crop on this family farm. However, in 2020, for the first time, sown soybean as a cover crop.

Sowing of cover crop was done after the bar-

ley harvest. The soil was prepared for soybean sowing and sowing was done shortly after the barley harvest, on June 26, 2020. Two very early varieties (00 maturity groups) were used in sowing: *Korana* (Agricultural Institute Osijek, Republic of Croatia) and *Merkur* (NS seeds, Serbia). Before sowing, the seeds treatment were bacterized – NB (NitrobakterinS, Faculty of Agrobiotechnical Sciences Osijek) preparation of mycorrhizal fungi was added – VAM+AC (VAM, Faculty of Agrobiotechnical Sciences Osijek), in combination with *Azotobacter chroococcum* bacteria.

Varieties belonging to 00 maturity group, ie very early maturity group, were used in the experiment. *Korana* variety has a purple flower color, yellow hair color and a dark brown hilum color. According to the height of the stem belongs to the medium-high varieties. The sowing norm for the *Korana* variety is 135 - 145 kg/ha, and the recommended set is 700,000 - 750,000 plants/ha. Genetic yield potential is above 4 t/ha. The oil content in the seeds of the *Korana* variety is 22-23%, and the protein content up to 42% (Agricultural Institute Osijek, 2020). Variety *Merkur* is also a very early variety (00 group). The genetic potential for yield is 5 t/ha. In addition to a stable and high yield, *Merkur* is resistant to lodging, which is recommended so that it can even be irrigated if it is post-sowing. *Merkur* variety has a stem of medium height that is overgrown with brown hairs. The seeds are of medium size with a yellow seed and a hilum of brown color. It can be grown either as the first crop or at later sowing dates, and it is also good as a cover crop after peas and barley because sowing can be done by the end of June. It can also be suitable for late regular sowing, for growing in mountainous areas. The optimal set is 550,000 plants per hectare.

#### *Weather conditions*

According to the data of the Croatian Mete-

orological and Hydrological Service (2020), the long term mean temperature (°C) by decades for the Gradište station (1999 - 2018) (Table 1) differed from the average air temperatures (°C) in 2020 by decades for Gradište station. The highest average air temperature (°C) in 2020 per decade for the Gradište station was in August (23.8°C). The highest average rainfall (mm) in 2020 per decade for the Gradište station was in June and averaged 113.1 mm, and the lowest average precipitation in April was 16.7 mm, while in the long term mean of rainfall (mm) per decade for the Gradište station (1999-2018) was the highest in June at 70.93 mm as considers to soybean vegetation period.

At the time of soybean germination in 2020, the highest precipitation fell in the 2<sup>nd</sup> decade of July 52.6 mm (Table 1). Later in vegetation, the amount of precipitation in 2020 was the highest in the 1<sup>st</sup> decade of August, 49.8 mm.

At the time of soybean germination in 2020, the average temperatures were 0.3 °C lower than the long term mean for Gradište station (1999-2018), and at the time of sowing in July 2020, the temperatures were for 1.3°C lower compared to the long term mean (Table 1).

#### *Collecting of plant material*

The harvest was done on 20<sup>th</sup> October, 2020. At the harvest, samples of plant material were taken to determine the components of soybean yield and to determine the yield per unit area.

Since the sowing of soybeans was performed at an inter-row spacing of 50 cm, samples were collected at 4 meters in length and from 3 rows (or repetitions) per treatment and separately for each variety. In this way, all plants were collected from one plot of 2 m<sup>2</sup> in 3 replications. All plants from one replicate that were collected from 4 m in length and from one replicate were placed in one bag next to the label.

Table 1: The mean air temperature (°C) and rainfall (mm) in soybean as cover crop vegetation period in 2020 by decades and the long term montly mean of Meteorological station Gradište (1999 – 2018) (Croatian Meteorological and Hydrological Service, 2020).

Decade	Air-temperature in 2020 (°C)				Rainfall in 2020 (mm)			
	June	July	August	September	June	July	August	September
I.	18.8	23.5	24.0	19.7	79.4	1.5	49.8	0
II.	20.3	19.8	23.9	20.9	8.1	52.6	1.1	0
III.	23.2	24.5	23.7	16.8	0	0	0	12.6
Average/Total	20.5	22.6	23.8	19.2	113.1	72.3	77.2	21.3
Long term mean of air-temperatures (°C)					Long term mean of rainfall (mm)			
Average/Total	21.1	22.9	22.5	17.3	84.28	64.5	50.1	66.4

Table 2: Analysis of Variance (ANOVA) of soybean yield components – model summary.

Source	DF	F-value	P-value	Significance
<i>Plant height</i>				
Genotype	1	10.06	0.002	$p < 0.01(**)$
Seed treatment	2	3.15	0.046	$p < 0.05(*)$
Genotype $\times$ Seed treatment	5	6.13	0.000	$p < 0.001(***)$
<i>Plant height to first fertile pod</i>				
Genotype	1	3.55	0.062	<i>ns</i>
Seed treatment	2	2.82	0.064	<i>ns</i>
Genotype $\times$ Seed treatment	5	3.82	0.003	$p < 0.01(**)$
<i>Number of pods per plant</i>				
Genotype	1	0.39	0.532	<i>ns</i>
Seed treatment	2	0.88	0.419	<i>ns</i>
Genotype $\times$ Seed treatment	5	2.31	0.048	$p < 0.05(*)$
<i>Seed mass per plant</i>				
Genotype	1	1.04	0.309	<i>ns</i>
Seed treatment	2	1.05	0.353	<i>ns</i>
Genotype $\times$ Seed treatment	5	3.71	0.004	$p < 0.01(**)$
<i>Thousand grain mass</i>				
Genotype	1	1.55	0.231	<i>ns</i>
Seed treatment	2	17.51	0.000	$p < 0.001(***)$
Genotype $\times$ Seed treatment	5	*	$< 0.001$	
<i>Seed yield</i>				
Genotype	1	9.99	0.006	$p < 0.01(**)$
Seed treatment	2	1.12	0.353	<i>ns</i>
Genotype $\times$ Seed treatment	5	*	$< 0.001$	

To determine the yield components from each treatment, 20 plants were selected and analyzed separately. A total of 120 individual plants were analyzed. The components of

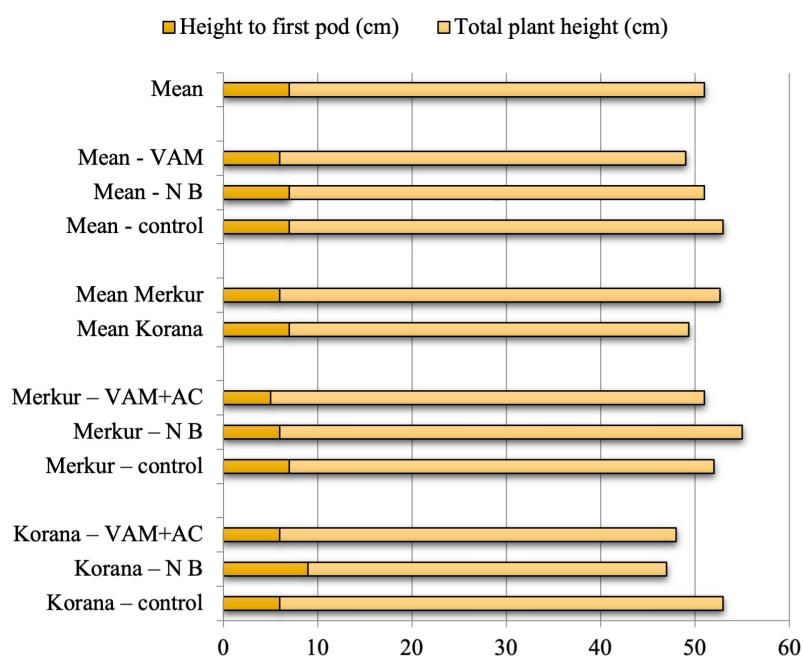


Figure 2: Plant height and height of first fertile pods of soybean cover crop in 2020 with bacteria and mycorrhiza seed pre-treatment.

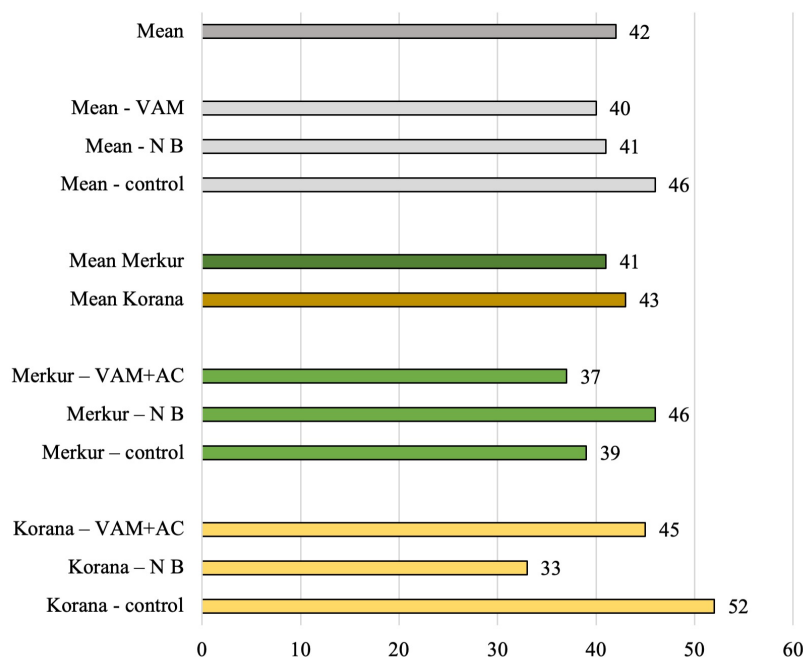


Figure 3: Number of pods per plant of two varieties depended of bacteria and mycorrhiza seed pre-treatment.

soybean yield were determined in the Laboratory for Analysis of Field Crops of the Faculty of Agrobiotechnical Sciences Osijek,

the following components were determined separately for each variety and for each treatment: plant height (cm), plant height to the

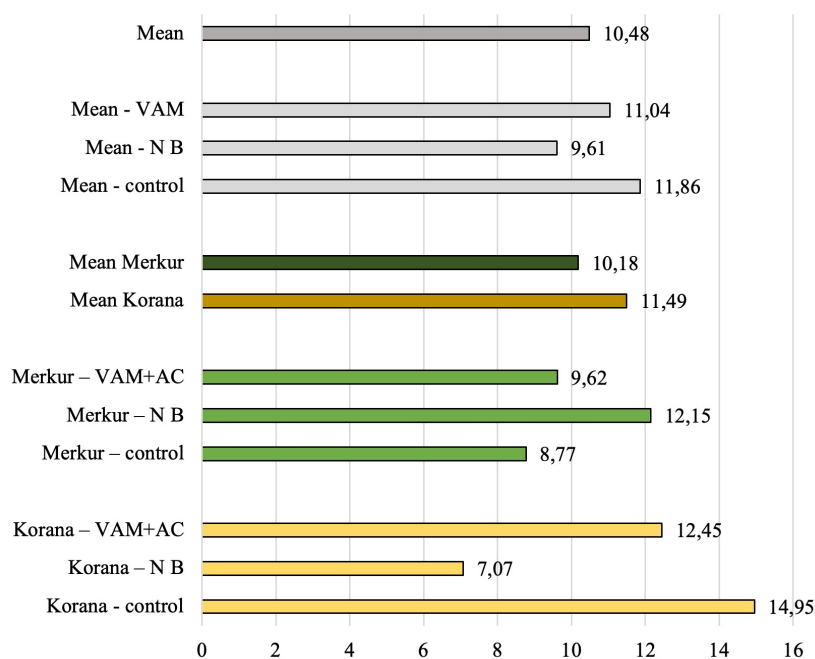


Figure 4: Seed mass per plant of two varieties depended of bacteria and mycorrhiza seed pre-treatment.

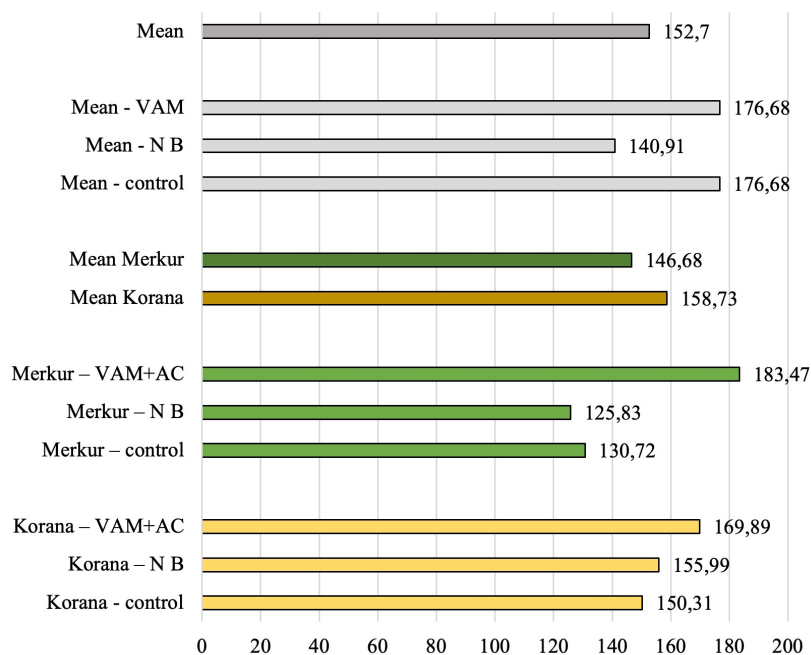


Figure 5: Thousand grain mass of soybean varieties depended of bacteria and mycorrhiza seed pre-treatment.

first pod (cm), number of pods per plant, determine soybean yield and calculated per mass of seeds of one plant and 1000 grains unit area. mass. The seeds of all plants were cleaned to

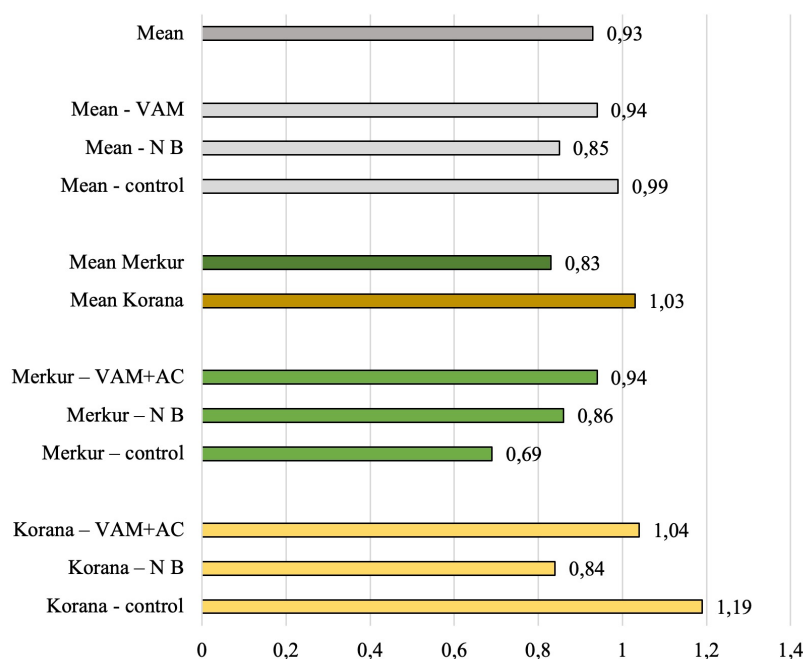


Figure 6: Soybean seed yield (t/ha) of two varieties depended of bacteria and mycorrhiza seed pre-treatment.

## Results

In this research, an analysis of the components of soybean soybean yield on the family farm "Alduk" in 2020 was made. The statistical analysis show different significance to each parameter (Table 2). According to certain yield components, the average plant height was 51 cm (Figure 2). According to the average of the variety only, the *Korana* variety had a stem with an average height of 49 cm, and the height to the first pod was on average 7 cm. According to the average of all treatments, the variety *Merkur* had an average stem height of 53 cm. According to the treatments, the highest stem had *Merkur* variety with the use of Nitrobacterin S (average 55 cm), while the lowest stem was with the *Korana* variety with the treatment with mycorrhizal fungi and *Azotobacter chroococcum*. The height of the plants up to the first fertile floor was on average 7 cm (Figure 2), and varied from 5 cm (*Merkur* variety with VAM + AC treatment), to 9 cm (*Korana* on

NB treatment).

Interestingly, the number of fertile pods per plant averaged 11 fertile levels on the main, central stem, so this data was not shown in the figures. However, when we look at the total fertile floors on a plant, on average the plants formed 16 fertile levels. The *Korana* variety had the least fertile floors on the plant in the treatment with Nitrobacterin (14), and the *Korana* variety had the most fertile floors again, but in the VAM + AC treatment.

The number of pods per plant (Figure 3) in this study averaged 42, with the seed weight of one plant being 10.48 g per plant (Figure 4). According to the average, the *Korana* variety had a slightly higher seed weight per plant (11.49 g per plant) compared to the *Merkur* variety (10.18 g per plant). The highest mass of seeds per plant had the strain of the *Korana* variety on the control treatment (14.95 g per plant). The *Korana* variety also had the lowest seed weight per plant (7.07 g per plant) with the application of NitrobacterinS.

The average mass of 1000 grains in this study was 152.701 g (Figure 5). According to the average variety *Korana* had a slightly higher weight of 1000 grains (average 158.73 g), while the variety *Merkur* had an average weight of 1000 grains 146.68 g. If we look at individual treatments, the highest mass of 1000 grains had the variety *Merkur* with VAM and treatment with mycorrhizal fungi and *Azotobacter chroococcum* (183.47 g).

The average soybean seed yield in this study was 0.93 t/ha (Figure 6). According to the conducted research, the variety *Korana* had an average yield of soybean seeds in post-sowing on the family farm "Alduk" in 2020 of 1.03 t/ha, while the variety *Merkur* had an average yield of 0.83 t/ha. If we look at the individual impact of the treatment, the *Korana* variety had the best yield on the control treatment (1.19 t/ha), then on the treatment with mycorrhizal fungi and *Azotobacter chroococcum* (1.04 t/ha), and the lowest with the application of NitrobakterinaS (0.84 t/ha). The *Merkur* variety had the lowest soybean yield on the control treatment (0.69 t/ha), while the addition of NitrobacterinS and VAM preparation with *Azotobacter chroococcum* soybean yield increased by about 19% with NitrobacterinS, and by about 27% with VAM and *Azotobacter chroococcum* (Figure 6).

## Discussion

Soybean as a cover crop can be produced for grain, silage, and in some conditions for green manure. For grain in lateral sowing, varieties of shorter vegetation 000, 00, and 0 maturity group are used at various inter-row sowing intervals depending on climatic conditions. Wang et al. (2011) state that soybean growth was significantly influenced by azotobacteria (*Glomus mosseae*) and rhizobium inoculation (*Bradyrhizobium* sp.), With higher dry matter mass and root-stem ratio being higher.

Siddiqui and Pichtel (2008) point out that mycorrhizae, which are indigenous organisms in the soil rhizosphere, have great potential in organic agricultural production. They have a positive effect on the growth of the root system, and can often control some plant pathogens. Fungal hyphae extend into the soil and secrete extracellular enzymes that efficiently absorb the maximum amount of nutrients available within root cells.

In our study, the Nitrobacterin treatment have positive for *Korana* variety, but it was not the same case for *Korana* variety. Milić et al. (2002) examined the potential for biological nitrofixation in eight different soybean genotypes with the aim of establishing a correlation between symbiotic community efficiency indicators and soybean grain yield. The results showed a positive correlation between grain yield, plant dry matter mass, nitrogen content in the aboveground part of the plant and in nodules, and nodule dry matter mass, from which they concluded that soybean grain yield depends not only on the efficiency of the micro symbiont but also on the genetic potential of the host plant. Differences between soybean genotypes in nitrofixation potential were also confirmed by Sudarić et al. (2008) who in their research also indicate a highly significant positive effect of bacterization on grain yield and soybean grain quality. Brevedan et al. (1978) found that in soybeans, with the increase in the amount of nitrogen available to the plant in the period from the beginning to the end of flowering, the grain yields also increased (in experiments in the greenhouse by 33% and in field experiments by 28-32%).

Keyser and Li (1992) write in their paper that a fully compatible symbiosis of legumes and nodule bacteria arises from the recognition, penetration, stimulation of host plant cells, differentiation of nodule bacteria into bacteroids, synthesis of leghemoglobin and nitrogenase, and nitrogenase activity. The amount of nitrogen bound by symbiotic ni-

trofixation varies considerably and can range from 0% to as much as 97% of the total nitrogen in the plant, but most estimates range from 25 to 75%. Biological nitrogen fixation is improved by selection to improve symbiotic nitrofixation, selection for the ability to nodulate and fix nitrogen in soils with high nitrogen content, development of soybean genotypes that have the ability to limit nodulation by indigenous strains of *B. japonicum*, but which allow nodulation by introduced by inoculation, and the development of soybean genotypes that form a symbiotic community with indigenous strains of *B. japonicum*.

Mycorrhizal fungi play a very important role in terrestrial ecosystems. They preserve the favorable structure of the soil, the circulation of matter in nature, regulate the cycles of carbon and other elements. In natural communities, mycorrhizae are thought to provide up to 80% of nitrogen and phosphorus to plants, and many plant species depend on this association.

According to Wani et al. (2013) bacteria of the genus *Azotobacter* synthesize auxins, cytokinins, and substances similar to gibberellic acid, which are the primary substances that control plant growth. These hormonal substances originating from the rhizosphere or root surface affect the growth of closely related higher plants. In order to guarantee the high efficiency of inoculants and microbiological fertilizers, it is necessary to find compatible partners, i.e. a certain genotype of the plant and a certain strain of *Azotobacter* that will make a good association.

## Conclusions

The aim of the study was to describe the importance of soybean seed bacterization and the use of mycorrhizal fungus preparations in post-sowing soybean as a cover crop in 2020

and to determine the impact of seed pre-treatment on yield and yield components. Two varieties *Korana* and *Merkur*, 00 maturity groups were used in the study.

The weather conditions in 2020 did not differ significantly from the multi-year average. The average height of the plant was 51 cm. According to the treatments, the highest stem had the strain of the *Merkur* variety with the use of Nitrobacterin S (average 55 cm), while the lowest stem was with the *Korana* variety with the treatment with mycorrhizal fungi and *Azotobacter chroococcum*. The height of the plants up to the first fertile pod was on average 7 cm, and varied from 5 cm (*Merkur* variety with VAM + AC treatment), to 9 cm (*Korana* on NB treatment). The number of pods per plant averaged 42, with the seed weight of one plant being 10.48 g per plant. In the case of the *Korana* variety, the average number of pods per plant increased by 3.20 for all treatments by increasing one fertile floor per plant, while in the *Merkur* variety the increase was smaller, and with the increase in the number of fertile floors per plant increased by 2.05.

The average soybean seed yield in this study was 0.93 t/ha. According to the conducted research, on average for all treatments, the variety *Korana* had an average yield of soybean seeds as cover crop in 2020 of 1.03 t/ha, while the variety *Merkur* had an average yield of 0.83 t/ha. *Merkur* variety had the lowest soybean yield on the control treatment (0.69 t/ha), while the addition of NitrobacterinS and VAM preparation with *Azotobacter chroococcum* soybean yield increased by about 19% with NitrobacterinS, and by about 27% with VAM and *Azotobacter chroococcum*.

Generally, probably due to low rainfall after sowing and in the summer period, the real effect of mycorrhizae and bacteria missed out due to lack of water.



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## Microbiological activities in the composting process – A review

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**Abstract:** Composting is a technological process of waste management that is, with the help of microbiological activities in aerobic conditions, organic material is decomposed and stabilized into a biodegradable mixture and transformed into compost. This process of decomposition of organic matter has recently attracted a lot of attention due to its environmentally friendly methods in which additional environmental pollution is avoided. The composting process follows four phases (first mesophilic phase, thermophilic phase, second mesophilic phase, and maturation phase). The most important factors influencing the decomposition success are C/N ratio, humidity, temperature, substrate particle size, pH, oxygen content and microorganisms. Microorganisms such as bacteria, fungi, and actinomycetes act as chemical decomposers in the process of decomposition of organic matter into carbon dioxide, heat, water, hummus, and a relatively stable final organic product - compost. In the process of composting, microorganisms decompose the complex molecules of lignin, cellulose, and hemicellulose. The presence of different types of microorganisms is influenced by the composition of composite mixtures and changes in temperature through the phases of the composting process. At the beginning of compression, the microbial activity increases significantly, which causes a temperature rise. The initial dominance of bacteria is replaced by fungi that are most active in the process of compost maturation. This scientific paper aims to present an overview of the composting process and the role of beneficial microorganisms in the process of decomposition of organic matter of the compost mixture.

**Keywords:** Microorganisms, compost, biodegradable material, microbiological degradation

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### Introduction

The composting process has been known since ancient times. The earliest evidence of the first composting dates back to the ancient Akkadian Empire, which used it to improve plant breeding (Vidović and Luttenberger, 2019). Waste today is one of the leading environmental problems of the modern way of life. An increasing amount of waste is generated due to the increase in human activities. Composting technology can be solution for reducing biodegradable waste, stabilization and environmentally friendly use and by-product recycling (Holes et al., 2014.). Today, there are many alternative ways to dispose of organic waste, and one of the ecological ways of disposing of biowaste is

composting, which involves the controlled oxidative microbiological decomposition of organic matter (Vargas-García et al., 2010). The resulting composts are organic fertilizers that are produced by the controlled microbiological decomposition of mixtures of fresh, dry, or processed plant residues, manure and organic waste from the processing industry, animal residues, and mineral additives (Lončarić et al., 2019).

Factors affecting the composting process include C/N ratio, humidity, oxygen, pH, temperature, and composition of all raw materials of the compost mixture (Kumar et al., 2010). The C/N ratio significantly affects the composting process because too wide ratio prolongs the composting process, and carbon deficiency causes nitrogen desta-

bilization and results in significant losses in ammoniac form (Wichuk and McCartney, 2010). Humidity is necessary for the chemical and microbiological processes in composting, and ideally the humidity during composting is 50-60%. The optimal oxygen concentration is 10%, while the ideal temperature depends on the phase in which the compost is (Bernal et al., 2009). The composting process consists of four phases; the first mesophilic phase, the thermophilic phase, and the second mesophilic phase, and the maturation phase of the compost (Sánchez et al., 2017).

The composting process as a biological process involves many microorganisms. It is the microorganisms that break down organic substances and organic compounds by their action and enzymes and turn them into a rich humic product (Albrecht et al., 2008; Aslam et al., 2008; Said-Pullicino et al., 2007). The microbiological population is affected by temperature, oxygen, humidity, nutrients, and pH reactions (Krstić et al., 2018). The most influential parameter on the presence of microorganisms in compost is the temperature (Steger et al., 2007). The starting components in compost also greatly affect the development of different microbial communities. The characterization of microbial communities during the composting process can provide important information on the development of the compost biodegradation process and on the maturity of the final product. Microbial activity is achieved by the action of enzymes responsible for the hydrolysis of complex macromolecules that make up the organic waste. The action of this type of enzymatic activity indicates the rate of decomposition of organic matter and the stability of the product (Mondini et al., 2004). Dehydrogenase activity indicates the index of biological activity due to its role in the oxidative process of phosphorylation (Vargas-García et al., 2010).

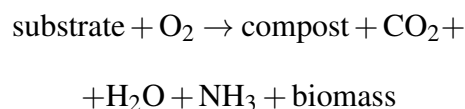
This scientific paper aims to present the com-

posting process, the role of beneficial microorganisms in compost conditioning, and the decomposition of organic matter in the compost mixture.

## Composting process

### *Field trail*

Composting is an aerobic microbiological process in which the decomposition of organic matter of materials found in initial mixtures occurs (Insam and de Bertoldi, 2007). The composting products produced by compost are gases (ammonia and carbon dioxide), water, and heat (Diaz et al., 2007). The composting process is also shown by the following equation (Haug, 2018):



The composting process is considered to be an environmentally friendly way of disposing of biological waste from the aspect of environmental protection with landfilling. Compost obtained only from biological waste can be used in organic agricultural production (Haug, 2018). Substrates used in composting are most commonly of plant, animal, or microbiological origin (Kuhad and Singh, 2007). The largest share is occupied by plant substrates, and animal and microbial components are occupied by smaller fractions in the compost mixture (Diaz et al., 2007).

The decomposition of complex structural aggregates is influenced by microorganisms, and their activity is present in certain phases of composting. The decomposition process begins with the oxidation of more easily degradable organic compounds, and this process is called putrefaction, followed by stabilization or decomposition of complex organic molecules and humification of lignocellulosic materials (Diaz et al., 2007).

### *Composting phases*

The composting process consists of four basic phases: the first mesophilic phase (25–45 °C), the thermophilic phase (45–65 °C), the second mesophilic phase (also called the cooling phase), and the maturation phase (Wichuk and McCartney, 2010).

The first mesophilic phase lasts on average several days then the temperature of the compost mixture increases above 40 °C after the 3rd day and thus the thermophilic phase begins. In this phase, soluble sugars and starches are broken down (Lončarić et al., 2015) under the influence of bacteria, fungi, and actinomycetes, which are called primary degraders by one name (Diaz et al., 2007). The number of mesophilic organisms in the compost mixture is three times higher than the number of thermophilic organisms, and the increase in temperature is due to their metabolic activity (Epstein, 1997).

The onset of the thermophilic phase depends on aeration, C/N ratio, and humidity (Vukobratović et al., 2008). This phase can last 10-30 days and is extended by mixing and additional wetting of the compost mixture (Lončarić et al., 2015). At this phase, decomposition acceleration occurs which increases until the temperature of the compost pile reaches a temperature of 62 °C (Tuomela et al., 2000). In the thermophilic phase, mixed populations of thermophilic bacteria and actinomycetes, and fungi that are tolerant to high temperatures develop. Under the influence of microbiological activities, the breakdown of proteins, fats, cellulose, and hemicellulose occurs (Sole-Mauri et al., 2007). Temperatures above 50 °C destroy the germination of weed seeds and pathogenic microorganisms. However, temperatures above 65 °C also destroy beneficial microorganisms, and then aeration of the compost pile can be useful or necessary (Lončarić et al., 2015). In the compost mixture, the temperatures in all parts are not the same, so for this reason it is important to reg-

ularly mix the compost mixture, which ensures that all parts of the compost are brought to the central part where the temperature is highest. The microbiological aspect indicates four zones of the compost mixture. The outer zone is well supplied with oxygen but has the lowest temperature, the inner zone is highly compacted and poorly supplied with oxygen, the lower zone has a high temperature and good oxygen supply, and the upper zone is the warmest and in most cases well supplied with oxygen (Figure 1) (Diaz et al., 2007).

The second mesophilic phase or cooling phase lasts variably which and depends on the initial mixture of the compost pile (Vukobratović et al., 2008). At this phase, mesophilic organisms are reactivated and long-term and slow degradation of lignin and other resistant components occurs (Huang et al., 2010).

In the compost maturation phase, the number of bacteria decreases, and there is an increase in fungi that break down agar and residual lignin (Diaz et al., 2007). This phase lasts from several days to several months, ie until the decomposition of carbon compounds takes place, and ends when stable and mature compost is obtained with a lower C/N ratio and a mild alkaline pH value (Abd El Kader et al., 2007).

### *Composting process factors*

The successful composting process depends on various physical, chemical, and biological factors, namely: substrate, particle size, humidity, temperature, pH, oxygen content, C/N ratio, and the number and type of microorganisms (Abd El Kader et al., 2007). It is important to have good starting materials for the composting process, and this usually includes fruit and vegetable residues, garden residues, kitchen waste, straw, manure, etc. In addition to plant residues, animal residues can also be mixed into the compost mixture. When making a compost mixture, it is important to choose good starting components that

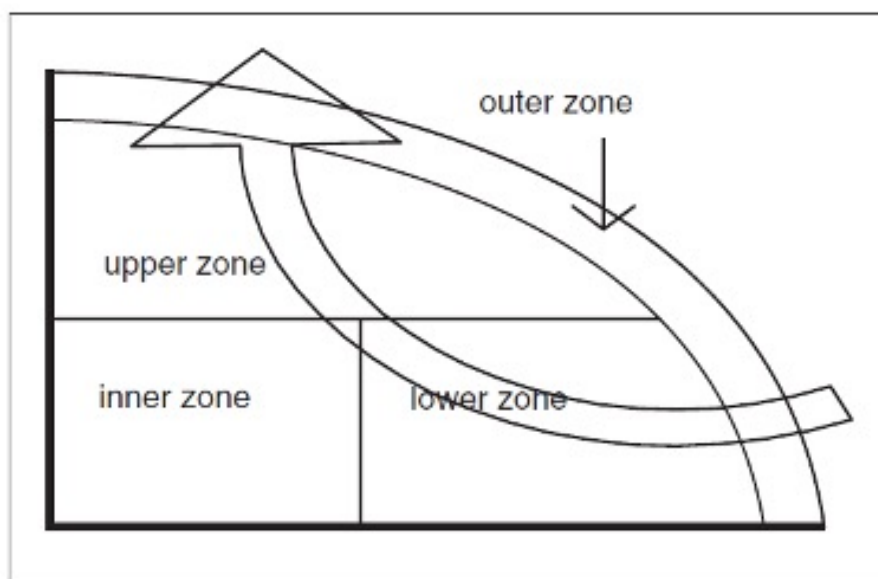


Figure 1: Compost piles zones. Source: (Diaz et al., 2007.)

contain enough nitrogen (green grass, green parts of plants, etc.), but also carbon (straw, sawdust, hay, cardboard, etc.) for the C/N ratio to be optimal (Malakahmad et al., 2017).

The optimal particle size of the initial components is 4–5 cm (Malakahmad et al., 2017). Tongneti et al. (2007) state that the initial components should be chopped to the size of an inch to increase the area available to microorganisms and speed up the composting process.

The ideal humidity at the beginning of the composting process is 50–60% (Castaldi et al., 2009). Excessive humidity above 70% affects the aeration of the compost mixture, i.e. reduces the air space and makes it difficult for oxygen to pass, which creates anaerobic conditions. The water content below 40% causes dehydration of the compost mixture and interruption of biological processes. If the moisture content of the compost mixture drops below 8% then all microbial activities cease (Abd El Kader et al., 2007).

The temperature of the compost mass rises rapidly above the ambient due to microbial activities. Composting is an exothermic process in which a large amount of en-

ergy is produced, but only about 45% of microorganisms are used to synthesize ATP, while the remaining energy is lost as heat in the compost mass (Diaz et al., 2007). The most active microbial activity is in the first mesophilic phase at a temperature of 30–45 °C (Majbar et al., 2018). Temperatures above 60 °C for at least 3 days are significant for sanitizing the compost pile and reducing pathogens. Lowering the temperature of the compost pile to the environment occurs at the end of the second mesophilic phase in which microbial activity decreases (Wang et al., 2013).

The optimal pH range for most actinomycetes and bacteria is 6.5–8.0, and this value corresponds to the final pH of mature compost. In the first mesophilic phase, the pH of the reaction is reduced because the decomposition of easily degradable organic materials from which organic acids are formed also occurs among the products (Kuhad and Singh, 2007). In the thermophilic phase, ammonia is formed due to the decomposition of the amine, which causes an increase in the pH of the compost mixture. In the second mesophilic phase, a

reduced pH occurs due to the reduced activity of microorganisms. In the maturation phase, the pH reaction stabilizes to a neutral value, which is closely related to the buffer capacity of humus (Yu and Huang, 2009).

It is important to provide a sufficient amount of oxygen to the microorganisms that carry out the decomposition of organic matter. In most cases, the oxygen content in the compost mixture at the beginning of the process is sufficient, however, to avoid anaerobic conditions during the composting process, an oxygen supply is required. Aeration of the compost pile is ensured by mixing or forced aeration by blowing air, and the optimal oxygen content for the activity of microorganisms must be greater than 10% (Guo et al., 2012). Oxygen content is highest during the thermophilic phase and decreases during the maturation phase because it slows down microbiological activity and releases carbon dioxide (Awasthi et al., 2016).

Carbon and nitrogen content is important in the composting process because microorganisms use carbon as a source of energy and nitrogen for cell construction and protein synthesis (Iqbal et al., 2015). The optimal C/N ratio in the composting process is considered to be 25/1 to 35/1 (Guo et al., 2012). If the C/N ratio is higher than optimal, the composting process is slowed down because the activity of microorganisms is reduced, and at a lower C/N ratio, ammonia and unpleasant odors are generated (Neugebauer et al., 2017). There is a possibility of a successful composting process even at values of C/N ratios that are lower than optimal, and the research of some authors is shown in table 1. Reduction of C/N ratios can be achieved by adding nitrogen-rich materials such as fruit and vegetable residues. Increasing the C/N ratio is achieved by adding raw materials with a higher carbon content such as cardboard and paper (Makan and Mounadar, 2012).

### **Microbiological processes in composting**

The biological circulation of nutrients is necessary for life, and the main mediators in that process are microorganisms. Biotransformation is a biological modification that changes the chemical structure of matter (Insam and de Bertoldi, 2007). Biotransformation can synthesize atoms or convert simple molecules into more complex compounds (biosynthesis) or vice versa (biodegradation and mineralization) (Michel et al., 2002).

#### *Microorganisms involved*

Microorganisms found in compost are mostly beneficial, however, some are potentially harmful to humans, animals, plants, and the environment (Fuchs, 2010). Harmful microorganisms are introduced mostly through the initial substrates of compost mixtures. Input substrates, compost pile size, rotation frequency, particle size, aeration, and wetting directly affect microbial processes (Fuchs, 2010).

Bacteria participate in biodegradation by producing carbon dioxide and heat to produce energy (Insam and de Bertoldi, 2007). The importance of non-mycelial bacteria during the composting process has long been neglected, probably due to better visibility of fungi and actinomycetes. Bacteria provide the fastest and most efficient composting by excreting nutrients, nitrogen, phosphorus, and magnesium (Abu-Bakar and Ibrahim, 2013). There are various types of bacteria in compost piles, where psychrophiles, mesophiles, and thermophiles predominate (Lee, 2016). Compared to other bacteria, psychrophilic bacteria secrete a small amount of energy and are most active at a temperature of 13 °C. Mesophilic bacteria are most active at a temperature of 21–32 °C and their role is similar to psychrophilic bacteria. When the temperature of the compost pile rises above 45 °C, the main role is taken over by thermophilic bacteria that con-

Table 1: Successful composting processes at lower C/N ratios

C/N ratio	Compost mix	References
15	Pig manure with wood sawdust	Huang et al., 2004
19,6	Waste from green areas and waste from the food industry	Kumar et al., 2010
20	Pig manure with rice straw	Zhu, 2007
20	Chicken manure with wood sawdust	Ogunwande et al., 2008

tinue to biodegrade in the composting process (Lee, 2016). For the genus *Bacillus*, the most optimal temperature is in the range of 50–65 °C, and at temperatures above 65 °C *Stearothermophilus* dominates, which is found in almost pure culture under such conditions (Insam and de Bertoldi, 2007).

Actinomycetes prefer a neutral or slightly alkaline pH of compost mixtures and participate in the degradation of more difficult to degrade substrates. Most actinomycetes thrive best when the compost pile is sufficiently moist and sufficiently supplied with oxygen (Insam and de Bertoldi, 2007). Actinomycetes can break down resistant materials such as starch and proteins while releasing carbon, nitrogen, and ammonia causing the earthy smell of compost (Shukla et al., 2014). Despite the lack of a nucleus, actinomycetes can grow multicellular threads such as spider nets and have enzymes that help break down cellulose, lignin, chitin, protein, woody stems, and tree bark.

Representatives of the *Thermus/Deinococcus* group grow on organic materials at temperatures of 40–80 °C, while their optimal temperature for growth is between 65 and 75 °C. *Thermus* species were once present only in geothermal sites and probably adapted to the hot compost system and play a major role at the peak heating phase of the compost mixture (Beffa et al., 1996). Many autotrophic bacteria have also been isolated from compost such as the *Hydrogenobacter* strain that was once also known only in geothermal sites (Insam and de Bertoldi, 2007).

Fungi get nutrients from dead plant mat-

ter, which is why they break down residues in compost, allowing bacteria to decompose even without cellulose (Lee, 2016). They form hyphae with which they penetrate the materials in the compost and thus decompose harder degradable substances such as lignin, hemicellulose, and cellulose (Nutongkaew et al., 2014). Fungi can break down dry and acidic residues and residues with low nitrogen content that are resistant to bacterial action. Complex polymers such as polyaromatic compounds or plastics are also degraded by fungi (Lee, 2016).

#### *Microorganisms at the beginning of the composting process*

At the beginning of the composting process, bacteria are most numerous, and fungi and actinomycetes are also important microbial community members. The composition of the microbial community in the compost pile is influenced by the starting materials (Klammer et al., 2008). All microorganisms present in the compost are found in a normal natural environment (Fuchs, 2010). The input components of the compost pile are often heterogeneous, and so are the initial microbial communities. Food waste containing plant residues has a low initial pH, which is why fungal and yeast proliferators are present and bacterial growth is slowed (Ryckeboer et al., 2003). Gram-negative,  $\alpha$ -,  $\beta$ -, and  $\gamma$ -proteobacteria were found primarily on compost samples containing leaves and grass on the first day of composting (Michel et al. 2002). Few mesophilic fungi and a large number of thermophilic bacteria and fungi



were found in the household waste (Ryckeboer et al., 2003). The presence of harmful organisms primarily depends on the input components of the compost mixtures. Animal waste from manure and food contains significant amounts of potential human and animal pathogens such as *Salmonella* sp., *Escherichia coli*, and *Listeria* sp. (Heinonen-Tanski et al. 2006, Wichuk and McCartney 2007; Grewal et al., 2007). Plant residues can also contain various plant pathogens. Soon after the onset of the composting process, the microbial population changes drastically and soon it no longer resembles the initial population (Fuchs, 2010).

#### *The succession of microorganisms during the composting process*

Shortly after the start of the composting process, microbiological biomass grows drastically (Fuchs, 2010). During the first day of composting, Klammer and Baath (1998) recorded a sixfold increase in microbiological mass in the composting of shredded straw with the addition of slurry. The physical and chemical properties of the compost mass change over time, and the microorganisms that are first active degrade the original substrate and produce metabolites and create a new physicochemical environment (Ryckeboer et al., 2003). One of the main factors influencing the microbial population in the composting process is the concentration and composition of dissolved organic matter (Fuchs, 2010). The main components of the organic matter of compost mixtures are proteins, carbohydrates, lipids, and lignin, and microorganisms produce different enzymes required for the degradation of different feedstocks (Ryckeboer et al., 2003). In the process of decomposition, bacteria dominate microbial communities, and during this phase, large amounts of organic carbohydrates are usually available in the compost mixture (Fuchs, 2010). The amount of nitrogen depends on the input raw materials,

and the optimal C/N ratio for the activation of microbial communities is 25–40. The activity of microbial communities causes an increase in the temperature of the compost mixture and a thermophilic phase occurs. It is at this phase that the greatest number of microbes and enzymatic activities occur (Cunha-Queda et al., 2007). As the temperature of the compost mixture increases, significant changes occur in the microbial community, which is important for the self-sterilization of compost, i.e. the destruction of harmful microorganisms (Fuchs, 2010). Different microbial communities were found in different places, which is related to different compost pile temperatures (Guo et al., 2007). The population of Gram-negative bacteria and fungi increased up to 50 °C but decreased at higher temperatures. After cooling, these two groups of microorganisms increased again (Ryckeboer et al., 2003). During the compost ripening phase, the number of bacteria decreases, but their diversity increases. At the same time, the fungi population is increasing in quantity and diversity (Ryckeboer et al., 2003). Fungal activity is expressed to be important in the compost ripening phase (Fuchs, 2010).

#### *Decomposition of organic matter in the composting process and the role of microorganism*

Lignocellulosic material consists of 38–50% cellulose, 23–32% hemicellulose and 15–25% lignin. In plants, it is also possible to identify structural polymers (waxes and proteins) that are represented by 5–13% (Deobald and Crawford, 1987). Lignocellulosic materials are by-products of various agro-industries and represent major problems in the environment. Various studies have examined the properties and methods of disposing of lignocellulosic materials such as sugarcane residues (García-Gómez et al., 2005), paper (Sung and Ritter, 2008), olive pomace (Komilis and Ham, 2003), tobacco waste,

(Pérez et al., 2002), leaves (Ekinci et al., 2000) and sawdust (Atkinson et al., 1996).

Lignin is the basic structural component of plants, and its degradation is the slowest compared to other components such as hemicellulose, starch, pectin and cellulose (Kuhad and Singh, 2007). The cause of slow degradation is due to the exceptional diversity of binding between monomer units (Ekinci et al., 2000). The biodegradation of lignin is mainly of the cometabolic type and therefore the amount of energy released is insignificant. Degradation of this polymer is most commonly achieved by white-rot fungi (*Stereum hirsutum*, *Phanerochaete chrysosporium*, *Trametes versicolor*) (Zeng et al., 2010). Some fungi, such as *Pleurotus ostreatus*, degrade both lignin and cellulose at the same time (Insam and de Bertoldi, 2007).

Cellulose is a polymer of glucose, which is made up of long rows of interconnected molecules of cellobiose disaccharides from which glucose is formed by complete hydrolysis (Diaz et al. 2007). Cellulose is rich in carbon and does not contain nitrogen, and fungi of mycelial structure (*Fusarium* sp., *Aspergillus* sp. and *Chaetomium* sp.) have a better ability to decompose (Insam and de Bertoldi, 2007). *Pseudomonas* and related genera are also known to degrade cellulose, and only a few actinomycetes are involved in this process (Sánchez et al., 2017).

Cellulolytic bacteria are ubiquitous and successfully degrade cellulose, while their ability to mineralize lignin is limited (Insam and de Bertoldi, 2007). *Cytophages* and *Sporocytophages* are the dominant cellulolytic microorganisms present in all phases of the composting process (Singh and Nain, 2014). Mesophilic aerobic and anaerobic forms of bacteria *Bacillus subtilis*, *B. polymyxa*, *B. licheniformis*, *B. pumilus*, *B. brevis*, *B. firmus* and *B. circulans* degrade hemicellulose (Singh and Nain, 2014).

Three types of fungi living on dead wood

found in compost mixtures, and these are soft rot fungi, brown rot fungi, and white-rot fungi (Singh and Nain, 2014). Soft rot fungi (Ascomycetes and Fungi imperfecti) effectively degrade cellulose, but only degrade slowly and incompletely the lignin content. Brown rot fungi (Basidiomycetes) show a tendency to break down carbohydrates and demethylate lignin. White rot fungi degrade both lignin and cellulose (Singh and Nain, 2014).

The composting process relies heavily on the function of decomposing organic matter by actinomycetes. Actinomycetes *Thermoactinomyces* and *Streptomyces* successfully decompose cellulose in the thermophilic phase of composting. The ability of actinomycetes to completely break down lignin is limited, but they extensively modify the structure of lignin with their enzymes (Singh and Nain, 2014).

Hemicellulose is a branched polymer of arabinose, xylose, glucose, and mannose. Together with lignin forms cross-linked structures that provide structural strength, but thus complicate the process of decomposition by microorganisms (Ladisch et al., 1983). Xylan is the most important among hemicellulose and is found in straw and the rest of sugar cane processing (up to 30%) and wood (2–25%). Xylan consists of pentose (xylose and arabinose) or hexose (glucose, mannose, and galactose) (Insam and de Bertoldi, 2007). The major enzymes for degrading, xylanase, are produced by many bacteria and fungi (Diaz et al., 2007). Pectin consists of unbranched polygalacturonic acid chains and is degraded by pectinase, which is common among fungi and bacteria (Insam and de Bertoldi, 2007).

Starch consists of amylose and amylopectin. Amyloses are unbranched chains of D-glucose, due to the position of the  $\beta$ -glycosidic bond, amylose is spiral, unlike cellulose. Amylopectin contains phosphate residues and magnesium and calcium ions

(Diaz et al., 2007).

Chitin is less important than cellulose, and the chemical composition of chitin is very similar to cellulose. The cellulose monomer is glucose and the chitin monomer is N-acetylglucosamine. The main difference for microorganisms that degrade chitin is that it contains a high concentration of nitrogen (about 7%), and the C/N ratio of chitin is approximately 5 (Park et al., 2005). Various fungi (e.g., *Aspergillus*) and bacteria (e.g., *Flavobacterium*, *Cytophaga*, *Pseudomonas*) use chitin as the source of nitrogen and carbon they need in degradation processes (Poulsen et al., 2008). Chitin is broken down through exoenzymes to N-acetylglucosamine, which is resorbed and broken down to fructose -6-P and is thus involved in carbohydrate metabolism (Insam and de Bertoldi, 2007).

## Conclusion

The composting process has been known since ancient times, but only in the last decades has more attention been paid to its importance in waste disposal. Transformation into compost of the biodegradable organic fraction of solid waste is one of the most validated methods of recycling. It is a process with low energy consumption and permits the disposal of the organic fraction of the solid waste which represent the greatest portion of refuse. Composting is the economically and ecologically most appropriate method of disposing of biological types of waste. Knowledge of the microbiological as-

pects of composting has permitted the optimization of all the factors which have a direct influence on the process. Optimal compost use in agriculture needs an appropriate determination of compost stability in relation to microbial activity. Stability prevents nutrients from becoming tied up in rapid microbial growth, allowing them to be available for plant needs. Microorganisms play an important role in the composting process because their enzymes break down the organic matter of compost mixtures. There is a complex interaction between different types of microorganisms, and their presence depends on the initial mixtures of the compost pile and the stage of the composting process. Although the outcome of a successful composting process is mostly known the interaction of all mechanisms and processes is still not sufficiently researched. Studying different types of microorganisms can help to discover more efficient and faster-composting models. It is for this reason that further research on microorganisms is important to achieve a better understanding of the composting and biowaste disposal process.

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## Control of plant pathogen *Fusarium* spp. with compost, compost tea application – A review

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**Abstract:** Compost has been used in agriculture for a long time for nutrient supply. However, in recent decades the disease suppressive effects of the compost and its aqueous extracts are in the focus of the research. Several composts and their water extracts were investigated on different plant diseases especially on those caused by soil-borne pathogens all over the world. The mechanisms are not fully understood. Disease suppression by compost and compost extract is attributed to various mechanisms like the presence of antagonistic microbes and unidentified chemical factors, induction of systemic resistance in plants. Probably the components of the studied substances trigger chemical and biological factors in plants. *Fusarium* species cause wide spectrum of plant diseases, therefore studies of their control are the hot spots of the researches. The control of *Fusarium* spp. was investigated in the processed literature based on the possible mode of action of composts or compost extracts in various experimental conditions as *in vitro*, greenhouse, pot and field experiments. The results proved to be very promising so far. In this respect, compost and compost tea could be possible alternatives to the synthetic chemical pesticides in controlling plant pathogens in a more environmentally sound way.

**Keywords:** Agro-waste, soil-borne pathogen, plant disease suppression, fungi

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### Introduction

Soil is inhabited by soil-borne pathogens, which could be a danger to crops. Therefore, broad spectrum of chemicals is widely used to control them for a long time now. These products are not specific, both pathogenic and non-pathogenic microbes are destroyed by their application. The most commonly used fumigants and other chemicals have proved to be harmful to people and the environment. It is therefore necessary to find alternative methods for controlling soil-borne diseases (Nicolopoulou-Stamati et al. 2016). According to the definition of International Biocontrol Manufacturers Association, the biocontrol agents or products have natural origin and limit the propagation of pests and pathogens. This is in accordance with the Di-

rective 2009/128/EC of the European Parliament and of the Council which promotes the non-chemical disease control.

The agricultural use of composts is an excellent form of minimising waste production, reducing environmental pollution and recycling of organic materials (Diaz et al. 2007), and is in line with the principles of circular economy (Jones and Comfort 2017). During compost application soil is enriched with nutrients and organic matter and, its structure could be improved (Diaz et al. 2007).

In the late 1970s, it was stated that compost can suppress plant pathogens and reduce disease incidence, especially the soil borne plant diseases (Hoitink 1980). From the last decades the number of papers dealing with controlling effects of water extract from compost against foliar diseases were pub-

lished (Yogev et al. 2010; Pane et al. 2012; Al-Mughrabi 2007).

Compost extracts were prepared by mixing compost and distilled water. Then the resulting slurry was incubated without agitation for a day (Koné et al. 2010; Bernal-Vicente et al. 2008). It can also be enriched with oxygen (Siddiqui et al. 2009). The slurry was filtered through cheesecloth, and the filtrate, termed an extract, was sprayed onto the aerial surfaces of plant or allocated into the soil (Kavroulakis et al. 2005; Bernal-Vicente et al. 2008). If the compost extract is effective in practice, it would be a potential alternative of fungicides and therefore, it could contribute to the sustainability of the agriculture (Yohalem et al. 1996).

In this review, the studies on composts and compost extracts against *Fusarium* spp. are in the focus. *Fusarium* species are imported pathogens in agriculture. They could cause significant damage during crop production. Different effective ways of compost, compost extract utilization for controlling plant diseases caused by *Fusarium* spp. were summarised and the future tasks of this scientific topic were revealed.

### **Effect of compost, compost tea application on plant pathogen *Fusarium* species**

Compost and compost tea were found as an environmentally sound option for controlling plant diseases. The genus *Fusarium* contains a number of soil-borne plant pathogenic species (Crous et al. 2021). Many cultivated plants may be attacked by different *Fusarium* species, e.g. *Fusarium oxysporum* damages tomato (Dukare et al. 2011) and carnation plants (Postma et al. 2003); *Fusarium solani* f. sp. *pisi* weakens the pea populations (Lumsden et al. 1983); *Fusarium oxysporum* f. sp. *radicis-cucumerinum* infects cucumber cultures (Bradley and Punja 2010). *Fusarium* species produce many mycotox-

ins (e.g. fusaric acid, trichothecene, marticin) and cause common root rot, wilt diseases, stem rot and necrosis in worldwide in the course of crop production (Crous et al. 2021; Jakucs and Vajna 2003).

The disease suppressive effects of compost and compost extract are examined all over the world and the number of studied compost varieties is almost unlimited. In addition, to generic ingredients such as different animal manure, straw, plant residues, composts often contain local raw materials for example Chinese medicinal herbal residues (Zhou et al. 2016; Shen et al. 2013), shrimp power and seaweed (Dionne et al. 2012), citrus waste (Bernal-Vicente et al. 2008; Lopez-Mondejar et al. 2010), olive waste (Ntougias et al. 2008; Basallote-Ureba et al. 2016). Moreover, spent mushroom substrate (Meng et al. 2018; Borrero et al. 2009; Tiltson et al. 2002) and sewage sludge (Lumsden et al. 1983; Heck et al. 2019) were also used to make compost. The results are promising. In most cases, *Fusarium* infection has been controlled by tested compost or compost extracts both in *in vitro* and *in vivo* treatments. However, in some cases inconsistent results were found. An interesting result was reported by Mierzwa-Hersztek et al. (2018) where compost extracts strongly reduced fungal sporulation, greater extent than the growth of fungi hyphae. In contrast to these results citrus compost and their extracts reduced significantly the mycelial growth of *Fusarium oxysporum* f.sp. *melonis*, but did not reduce the spore germination in *in vitro* experiments (Bernal-Vicente et al., 2008). Compost extracts had *in vitro* inhibitory effects on the tested *Fusarium* while no *in vivo* inhibitory effects were found (Znaïdi et al. 2002). De Corato et al. (2016) and Bernal-Vicente et al. (2008) reported suppressive results both in *in vitro* and *in vivo* investigations.

Summary of works on the suppressing effects of composts and their extracts on

*Fusarium* species are presented in Table 1.

### **Mechanisms of action of composts and compost extracts**

It is very difficult to separate the different mechanisms of action. Microbes in the compost effect in various ways against soil pathogens (antibiosis, parasitism, microbiostasis), but only some of the real possibilities could be in focus in individual experiments. Researchers suggested the most probable implementations based on their results. To approach a problem from several aspects could result in finding more opportunities for effective implementation. The different possible mechanisms are detailed in the following subchapters.

#### *Microbe content of compost/compost tea*

Biotic components of extracts play a significant role because some compost lost partially their suppressiveness after sterilization (Cotxarrera et al. 2002; EL-Masry et al. 2002). The sterilization by autoclaving or by microfiltration ceased inhibition effect of compost on the mycelial growth of the tested pathogens (Koné et al. 2010). During microfiltration not only microorganisms were filtered out, but also their by-products or metabolites (Siddiqui et al. 2009). EL-Masry et al. (2002) found that all autoclaved compost water extracts lost their antagonistic effects against the indicator fungi because autoclaving kills all sporulated and non-sporulated microorganisms. Xiong et al. (2017) pointed out a possible new role of some keystone species (e.g. *Trichoderma* and *Bacillus* spp.) of composts in modification of structure and function of indigenous microbial groups of soil.

#### *Competition for resources*

Microorganisms living in the same niche compete for nutrients and places. The most

widely known form is siderophore production of *Pseudomonas* sp. which is effective against *F. oxysporum* f. sp. *dianthii* (Duijff et al. 1993). Larkin and Fravel (1998) also found the nutrient competition as the main effect against *Fusarium* infection on potato plants. Competition effect could be increased by the increasing number, richness and diversity of bacterial strains in soil after compost treatment (Fu et al. 2017).

#### *Antagonism, antibiosis and parasitism*

Antagonism means that one organism suppresses or interferes the normal growth and activity of another organism via different mechanism. Antibiosis is a process in which toxic metabolites can penetrate into the cell and inhibit its activity by chemical toxicity while in the course of parasitism the parasitic organism acquire some or all of their feed stuff requirements from other organisms. Fungal antagonists added to composts in different maturation ages could increase the disease suppressiveness against *Fusarium oxysporum* by the antagonist enrichment (Postma et al. 2003). However, in this case not only the original microbial community of the compost extract was used even so the experiment proved the effect of antagonism. The most probable antagonistic mechanism of these bacterial mixtures depends on the lysogenic activity of bacteria but it needs a direct contact between the bacteria and the phytopathogenic fungi (EL-Masry et al. 2002).

Mycoparasitism is a special type of parasitism when fungus attacks another fungus (e.g. *Trichoderma* sp. against *F. oxysporum*) (John et al. 2010). Other, widely used antagonistic microbes are within *Pseudomonas* (Wahyuni et al. 2010) and *Bacillus* (Dukare et al. 2011) genus.

#### *Induced resistance in plants*

Certain infection may activate induced resistance in plant. Two forms of induced resistance are known. Systemic acquired resis-

tance (SAR) which are associated with a salicylic acid-dependent signalling pathway and the induction of pathogenesis-related (PR) proteins. In contrast, induced systemic resistance (ISR) are not salicylic acid dependent and it can be induced by defined bacterium taxa. Induced resistance in plants can be induced by soil or foliar application of compost tea in controlling foliar and soil-borne pathogens (Zhang et al. 1998; Yogeve et al. 2010; Kavroulakis et al. 2005). Therefore, the application of compost either soil amendment or foliar spray may be effective to improve the level of disease suppression in fields (Joshi et al. 2009). Molecular mechanism liable for compost-induced systemic acquired resistance is not fully understood so far but it can be concluded that compost-induced SAR differed from SAR induced by pathogens (Zhang et al. 1998).

However, microorganisms also play an important role for inducing resistance. Aimé et al. (2013) found that a non-pathogenic *F. oxysporum* F047 strain induces the systemic resistance of tomato plants against *F. oxysporum* f. sp. *lycopersici*.

#### *Chemical factors*

Compost extract contain biocontrol agents as well as unidentified chemical factors which play a role in different pathogenic fungal suppression (Cronin et al. 1996). High pH and high electrical conductivity (EC) of compost may affect the extent of the suppressive effect of the diseases (Lamprecht et al. 2017; Cotxarrera et al. 2002). Pathogens need different microelements for growth and sporulation. High pH and EC reduce these microelements availability hereby can decrease the virulence of plant pathogens (Cotxarrera et al. 2002; Amir and Riba 1990). Different nitrogen forms may be an influencing factor in the mechanism of action (Trillas-Gay et al. 1986; Tiltson et al. 2002). Calcium nitrate and ammonium sulphate content of compost significantly reduce the disease

severity, on the other hand ammonium nitrate nitrogen had no effect of *Fusarium* wilt of radish (Trillas-Gay et al. 1986). Heck et al. (2019) studied the abiotic and biotic characteristics of compost-treated soil and concluded that the most consistent abiotic factors were EC pH, base saturation%, cation exchange capacity, P, K, Ca, Mg, Na, Zn, Mn and B.

#### *Multiple effects*

Combination of physico-chemical and biological characteristics of composts for suppressing plant diseases (Kavroulakis et al. 2005; Siddiqui et al. 2009; Zmora-Nahum et al. 2008) can be the real explanation for antifungal effect of compost/compost tea. Physico-chemical characteristics include physical and chemical aspects of compost that reduce disease severity by directly affecting the pathogen or host capacity for growth. Examples of these aspects include nutrient levels, organic matter, moisture, pH (Cronin et al. 1996), and other factors (Cotxarrera et al. 2002). Kavroulakis et al. (2005) reported that the biological control of pathogens by compost depends on both biological and chemical factors. It is actually a complex and difficult process.

#### **Effects of compost of different origins**

Several studies investigated the effects of different composts. Disease suppression was significantly affected by storage method, composition of compost samples, as well as their interaction which indicate that the effect of the storage on disease suppression is compost-dependent (van Rijn et al. 2007; Saadi et al. 2010; Postma et al. 2003). Termorshuizen et al. (2006) did a large comparative assessment. The impacts of 18 different composts were examined on seven pathosystems among them *Fusarium oxysporum* f. sp. *lini*. None of the composts showed significant disease suppression against all

pathogens. The second highest disease suppression was found for *Fusarium oxysporum*. 14 composts of the examined 18 composts suppressed by more than 50% of the disease caused by the pathogen. At least one compost induced a disease suppression by more than 70% for each pathosystem. Similar results were published by Pane et al. (2011). They suggest that compost suppressiveness is often pathogen specific. Only one of the tested composts suppressed significantly all the studied pathogens. Probably there is no compost which could suppress all or many pathogens. However, if the suppressive properties of the compost are known, it can be applied in practice.

Ntougias et al. (2008) studied nine composts mixed with peat (after curing and following nine month storage) and assessed for their suppressive effects against two major soil-borne pathogens of tomato. The studied pathogens show great phylogenetic distance, had different life cycles and mode of action. Disease incidence in tomato by *Phytophthora nicotianae* was significantly reduced by all studied compost amendments. On the other hand, the effects of these compost applications for the protection from *Fusarium oxysporum* f.sp. *radicis-lycopersici* infection presented higher variability. Fresh composts usually caused significantly lower disease incidence values than prolonged stored composts.

Based on these results we can conclude that each compost have to be tested for their effectiveness against different pathogens.

### Conclusion and future possibilities

Agriculture has to satisfy the qualitative and the quantitative demands of the consumers. Compost and aqueous compost extracts still have some untapped potentials. They can be produced from waste materials in an inexpensive way. However, large amounts are spent on pesticides in agricul-

ture. The question is how can decrease the quantity of pesticides by good quality composts and compost teas having proved effect against well-defined pathogens based on well-defined application technology. Application of composts and compost extracts improve soil quality by altering its physical, chemical and microbiological properties. They increase the organic matter content, water capacity, microbial species diversity and macro- and micro-nutrient supply of soils, which are fundamental for plant growth. In addition, application of composts as soil amendments and foliar sprays may be an effective way to improve the level of disease suppression in field situations. Many pathogenic fungi can induce disease in field plants, including members of the genus *Fusarium*. The main shortcoming of the field applications of compost extracts are the lack of standardization of preparation and application methods.

Only some recent investigations assessed the suppressiveness of many compost types on different pathogens. Results of the studies also suggest that compost suppressiveness is often pathogen specific. Generally, there was not compost which had significant disease suppression against all the examined *Fusarium* sp. and pathogens, in addition the pathogens were not affected similarly by all composts. Moreover, big differences in compost quality and their impact on plant health were observed. This may be due to the different microbial composition of the composts. Microbes have a major role in compost-mediated disease suppression and since composts from different organic sources vary notably in their microbial composition, these differences may result in variable disease suppression, as well.

One of the tasks is to find the best compost or compost extract for the selected *Fusarium* sp. or other pathosystem, thereby it contributes to reducing the risk of chemicals used in agriculture.

Another task is to find the true mode of action. It is quite difficult to separate the different mechanisms of actions. Studies usually focus only one action the researcher assumed. In fact, it is a very complex process therefore, more complex and even more studies of composts and aqueous compost extracts are needed.

The following tasks are the hot points of this research area: i) chemical examination of a wide range of the extracts, ii) detailed analysis of the microbial community of the extracts, iii) investigation of the effects of

isolated microbes against *Fusarium* sp. and different plant pathogens both *in vitro* and in field experiments, iv) standardisation of composting process, compost tea producing process and the application method of the compost tea, v) storage of effective compost teas. After completing these tasks, not only the suppressiveness of composts and compost extracts could be used for the reduction of pesticides applied in agriculture but this method is also one of the most cost effective, environmentally sound alternative for organic waste recycling.

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Table 1: Suppressing effects of compost and their extract on *Fusarium* species in different experimental conditions.

Tested pathogen	Compost materials	Tested material	<i>in vitro</i> /plant	Effects	Supposed mechanism	References
<i>Fusarium culmorum</i>	Green waste, paper waste, sewage sludge, spent mushroom, horse manure, municipal solid waste.	Compost	Winter wheat	Two of eight tested composts were suppressive.	Pathogen specific; abiotic component.	Tilston et al. 2002
	Rape straw, wheat straw, chipped maize straw, fragmented waste material obtained during shelling of pea seeds, ground polymer materials, which were made of polyethylene and thermoplastic corn starch.	Compost extract	<i>in vitro</i>	Compost extracts strongly reduced fungal sporulation, greater extent than the growth of fungal hyphae.	Heavy metal.	Mierzwa-Hersztek et al. 2018
<i>Fusarium foetens</i>	Commercial compost.	Compost	Rooibos, oat, lupin	Both composts significantly suppressed damping-off caused by <i>Fusarium</i> spp.	Biological component; abiotic factors.	Lamprecht and Tewoldemedhin 2017
<i>Fusarium graminearum</i>	Cattle manure, sheep manure, chicken manure, horse manure, vegetable waste, ground straw.	Compost extract	<i>in vitro</i>	Inhibitory effect on the mycelial growth of fungus.	Role of microorganisms.	Kerkeni et al. 2007
	Rape straw, wheat straw, chipped maize straw, fragmented waste material obtained during shelling of pea seeds, ground polymer materials, which were made of polyethylene and thermoplastic corn starch.	Compost extract	<i>in vitro</i>	Compost extracts strongly reduced fungal sporulation, greater extent than the growth of fungal hyphae.	Heavy metal.	Mierzwa-Hersztek et al. 2018
<i>Fusarium oxysporum</i>	Horticultural waste, prune, cut grass, ditch plants, fruit, vegetable and garden waste with different maturation stages.	Compost	Carnation	Disease suppression.	Origin of compost; antagonism.	Postma et al. 2003
	Cattle manure, sheep manure, poultry manure, crushed wheat straw.	Compost extract	<i>in vitro</i> and potato	<i>In vitro</i> : all compost extracts had inhibitory effects. <i>In vivo</i> : no inhibitory effects.	Microorganism content of compost tea.	Znaïdi et al. 2002
	Paddy straw.	Composts and their extracts	Tomato	Severe reduction in pre- and post-emergence disease incidence.	Interactions among various modes of biocontrol by microorganisms.	Dukare et al. 2011
	Commercial compost.	Compost	Roiboos, oat, lupin	Both composts significantly suppressed damping-off caused by <i>Fusarium</i> spp.	Biological component; abiotic factors.	Lamprecht and Tewoldemedhin 2017

Table 1: (continued): Suppressing effects of compost and their extract on *Fusarium* species in different experimental conditions.

Tested pathogen	Compost materials	Tested material	<i>in vitro</i> /plant	Effects	Supposed mechanism	References
<i>Fusarium oxysporum</i>	Green waste	Compost extract	<i>in vitro</i>	The colony diameter of <i>F. oxysporum</i> was reduced to a small extent.	Microbial populations present in compost extract.	Milinković et al. 2019
	Chinese medicinal herbal residues, food waste (rice, bread, cabbage, boild pork), sawdust.	Compost extract	<i>in vitro</i>	Acetone extract of the compost showed the best performance against fungal growth. Antagonist properties of mature compost were stronger.	Antagonism; mycoparasitism.	Zhuo et al. 2016
<i>Fusarium oxysporum</i> f. <i>sp. basilici</i>	Cow manure, wheat straw, chicken manure.	Compost	Sweet basil	Significant reduce of disease severity.	Biotic factors.	Reuveni et al. 2002
	Agricultural and agro-industrial residues, plant green waste, municipal solid waste, cattle manure and other biowaste.	Compost	Basil	All green composts were capable of effectively suppressing the pathosystems under consideration.	Biological activity of composts' microbial community.	De Corato et al. 2019
<i>Fusarium oxysporum</i> f. <i>sp. conglutinans</i>	Canadium shangum peat, perlit	Compost	Radish	Unheated compost media significantly suppressed the population of the tested pathogen.	Biotic and abiotic factors.	Trillal-Gay et al. 1986
<i>Fusarium oxysporum</i> f. <i>sp. cubense</i>	Pig manure, cattle manure, Chinese medicine residue.	Compost	Banana	Cattle manure compost and Chinese medicine residue compost were the most effective.	Enzymatic and microbiological parameters; increased soil bacterial and actinomycetes populations.	Shen et al. 2013
	Sewage sludge.	Compost extract	Banana	The aqueous extracts of sewage sludge reduced fungal microconidial germination. Sewage sludge increased mycelial growth when compared with control.	Both biotic and abiotic characteristics.	Heck et al. 2019
<i>Fusarium oxysporum</i> f. <i>sp. cucumerinum</i>	<i>Caragana microphylla</i> -straw, chicken manure.	Compost	Cucumber	The number of <i>F. oxysporum</i> was significantly decreased by <i>C. microphylla</i> -straw compost application.	Microbial diversity.	Tian et al. 2016
<i>Fusarium oxysporum</i> f. <i>sp. dianthi</i>	Grape marc, cork, olive oil husk, cotton gin trash, rice husk, spent mushroom compost, peat, coir fibre, vermiculite.	Compost	Carnation	Grape marc compost was the most effective. Coir fibre, peat and vermiculite were conductive for this disease.	Microbiostasis.	Borrero et al. 2009
	Grape marc.	Compost	Tomato and carnation	Significant interaction was found.	Disease parameters affected by the water use of plants.	Sant et al. 2010



Table 1: (continued): Suppressing effects of compost and their extract on *Fusarium* species in different experimental conditions.

Tested pathogen	Compost materials	Tested material	<i>in vitro</i> /plant	Effects	Supposed mechanism	References
<i>Fusarium oxysporum</i> Schl f. sp. <i>lactucae</i>	Commercial compost.	Compost	Lettuce	Compost treatments were not suppressive in all the trials.	Microbial inhibitors.	De Corato et al. 2011
<i>Fusarium oxysporum</i> f. sp. <i>lini</i>	Horse manure, green waste, tree bark, slurries, urban biowastes, organic residues of wine grapes, woody wastes, poultry manure, woodcut, plants, spent mushroom, leonardite, urea, phosphate, catalyst, wood chips, municipal sewage sludge, yard waste, manure clay, plant residues - from four countries.	Compost	Flax	High disease suppression.	Pathogen specific effects but the mechanisms are not identified.	Termorshuizen et al. 2006
	Vegetable, fruit and garden waste, leaves and wood trimming, manure, clay.	Compost	Flax	75% of the assays resulted in significant disease suppression. Disease suppression was significantly affected by storage method.	Bacterial composition and microbial activity. Storage have a limited effect.	van Rijn et al. 2007
<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>	Green waste.	Compost	Tomato	Significantly reduced <i>Fusarium</i> wilt symptoms.	Antagonism; competition for space and nutrients.	Cucu et al. 2020
	Agricultural and agro-industrial residues, plant green waste, municipal solid waste, cattle manure and other biowaste.	Compost	Tomato	All green composts were capable of effectively suppressing the pathosystems under consideration.	Biological activity of composts' microbial community.	De Corato et al. 2016
	Miscanthus, giant reed, wheat straw, agro-industrial co-products (coffee-ground, defatted olive marc, woodchip), plant-waste (artichoke, fennel, tomato).	Composts and their extracts	<i>in vitro</i> and tomato	Both <i>in vitro</i> and <i>in vivo</i> the suppression of the tested pathosystem was highly significant.	Pathogen-specific; compost microflora; antibiotic mechanisms.	De Corato et al. 2016
	Commercially available compost (vegetable and animal wastes, sewage sludge, yard waste)	Compost	Tomato	The compost effectively suppress <i>Fusarium</i> wilt.	Biotic and abiotic factors.	Cotxerrera et al. 2002

Table 1: (continued): Suppressing effects of compost and their extract on *Fusarium* species in different experimental conditions.

Tested pathogen	Compost materials	Tested material	<i>in vitro</i> /plant	Effects	Supposed mechanism	References
<i>Fusarium oxysporum</i> f. sp. <i>melonis</i>	Citrus wastes, sludge obtained from citrus industry waste water treatment, green residues - in two proportions.	Composts and their extracts	<i>in vitro</i> and melon	Both citrus composts and their water extracts reduced significantly the mycelial growth, but did not reduce the spore germination in <i>in vitro</i> experiments. The <i>in vivo</i> assay confirmed the <i>in vitro</i> results.	Antagonistic microorganism; mycoparasitism (lytic enzyme).	Bernal-Vicente et al. 2008
	Commercial compost.	Compost	Melon	No suppressive effect.	-	De Corato et al. 2016
	Citrus wastes, sludge obtained from citrus industry waste water treatment, green residues.	Composts and their extracts	Melon	Pathogen incidence was significantly lower in than peat. In case of water extracts, one of them showed lower, other of them showed higher pathogen incidence than its solid matrix.	Biotic (antibiosis and mycoparasitism) and abiotic components; niche competition or plant acquired and/or induced resistance.	Lopez-Mondejar et al. 2010
	Sewage sludge.	Compost	Melon	Significant decrease in disease was observed.	Microorganisms of the compost.	Lumsden et al. 1983
	Cow manure, tomato plants.	Compost	Melon	All composts were highly suppressive and clearly effective compared to the peat in the course of period.	Microbial populations of compost; induced systemic resistance; storage had no negative effect.	Saadi et al. 2010
	Horticultural waste mixture, pepper plant waste, almond peel waste.	Compost	<i>in vitro</i>	1.4% of the selected microorganisms from different composts against <i>F. oxysporum</i> could inhibit the growth of the pathogen.	Compost maturity; antagonistic microorganisms.	Suárez-Estrella et al. 2007
	Ground tomato-plant residues mixed with separated cattle manure.	Compost	Melon	When both parts of the root system were grown in compost, disease severity was further reduced.	Induced resistance.	Yogev et al. 2010
	Vineyard pruning waste.	Compost	<i>in vitro</i> and muskmelon	In both cases compost showed higher biocontrol activity than peat against <i>Fusarium oxysporum</i> .	Biotic and abiotic characteristics of the compost.	Blaya et al. 2013

Table 1: (continued): Suppressing effects of compost and their extract on *Fusarium* species in different experimental conditions.

Tested pathogen	Compost materials	Tested material	<i>in vitro</i> /plant	Effects	Supposed mechanism	References
<i>Fusarium oxysporum</i> f. sp. <i>melonis</i>	Miscanthus, giant reed, wheat straw, agro-industrial co-products (coffee-ground, defatted olive marc, woodchip), plant-waste (artichoke, fennel, tomato).	Composts and their extracts	<i>in vitro</i> and melon	Both <i>in vitro</i> and <i>in vivo</i> the suppression of the tested pathosystem was highly significant.	Pathogen-specific; compost microflora; antibiotic mechanisms.	De Corato et al. 2016
	Steam-explosion liquid waste (giant reed, <i>Miscanthus sinensis</i> , Kenaf, wheat/barley straw), agro-industrial residues (defatted olive marc, coffee ground, wood chip, aspen chip, viticulture and vinery residue), plant green-waste (artichoke, fennel, tomato, escarole, potato, pepper), municipal solid waste, cow manure, household waste.	Composts and their extracts	<i>in vitro</i> and melon	Suppressive activity of composts and their extracts depending the feedstock origin both <i>in vitro</i> and <i>in vivo</i> assay.	Pathogen-specific; compost microbiota (depend on feedstock).	De Corato et al. 2018
	Agricultural and agro-industrial residues, plant green waste, municipal solid waste, cattle manure and other biowaste.	Compost	Melon	All green composts were capable of effectively suppressing the pathosystems under consideration.	Biological activity of composts microbiomes.	De Corato et al. 2019
<i>Fusarium oxysporum</i> f. sp. <i>radicis-cucumerinum</i>	Tomato, pepper plant waste, solid material separated from dairy farm liquid manure, pine bark.	Compost	Cucumber	Two composts significantly reduced the disease severity, while one compost had no effect.	Antibiotic production.	Bradley & Punja 2010
	Raw pruning residues, sewage sludge, sawdust, winery residues including grape stalks and grape pomace, tomato pulp with sawdust, organic fraction of municipal solid waste, olive mill extracted press cake, waste water, olive leaves.	Compost	Cucumber	Four of the examined six composts were significantly suppressed root and stem rot symptoms on cucumber.	Phenolic compounds.	Markakis et al. 2016
<i>Fusarium oxysporum</i> f. sp. <i>radicis-lycopersici</i>	Grape mark waste, extracted olive press cake, peat.	Compost extract	Tomato	Increased plant resistance on peat amended with compost.	Combination of physico-chemical and biological characteristics of compost; systemic resistance.	Kavroulakis et al. 2005
	Cattle manure, sheep manure, chicken manure, horse manure, vegetable waste, ground straw.	Compost extract	<i>in vitro</i>	Inhibitory effect on the mycelial growth of the tested fungi.	Microorganisms.	Kerkeni et al. 2007

Table 1: (continued): Suppressing effects of compost and their extract on *Fusarium* species in different experimental conditions.

Tested pathogen	Compost materials	Tested material	<i>in vitro</i> /plant	Effects	Supposed mechanism	References
<i>Fusarium oxysporum</i> f. sp. <i>radicis-lycopersici</i>	Grape mark waste, spent mushroom compost, olive tree leaves, olive wastewaters, olive press cake, extracted olive press cake.	Compost	Tomato	Most of compost amendments reduced the disease severity in	Systemic resistance.	Ntougias et al. 2008
	Sphagnum peat moss with composted pulp and paper mill residues, peat.	extract Compost	Tomato	varying degree. growth of the tested fungi. Markedly reduced symptom severity.	Induced resistance.	Pharand et al. 2002
	Bovine manure, chicken manure, sheep manure, shrimp powder, seaweed.	Compost extract	<i>in vitro</i>	Reduced mycelial growth.	Microbial communities; induction of plant defence reactions by microorganisms or by organic and inorganic compounds of the compost tea.	Dionne et al. 2012
<i>Fusarium roseum</i> var. <i>graminearum</i>	Cattle manure, sheep manure, poultry manure, crushed wheat straw.	Compost extract	<i>in vitro</i> and potato	<i>In vitro</i> : all compost teas had inhibitory effects. <i>In vivo</i> : no effects.	Microorganism content of compost tea.	Znaïdi et al. 2002
<i>Fusarium roseum</i> var. <i>sambucinum</i>	Cattle manure, sheep manure, poultry manure, crushed wheat straw.	Compost extract	<i>in vitro</i> and potato	<i>In vitro</i> : inhibitory effects. <i>In vivo</i> : no effects.	Microorganism content of compost tea.	Znaïdi et al. 2002
<i>Fusarium solani</i>	Cattle manure, sheep manure, chicken manure, horse manure, vegetable waste, ground straw.	Compost extract	<i>in vitro</i>	Inhibitory effect on the mycelial growth of fungus.	Role of microorganisms.	Kerkeni et al. 2007
<i>Fusarium solani</i> f. sp. <i>pisi</i>	Sewage sludge.	Compost	Pea	Disease was increased by compost.	Microorganisms of compost stimulated the soil microbial community.	Lumsden et al. 1983
<i>Fusarium solani</i> var. <i>cæruleum</i>	Cattle manure, sheep manure, poultry manure, crushed wheat straw.	Compost extract	<i>In vitro</i> and potato	<i>In vitro</i> and <i>in vivo</i> : inhibitory effects.	Microorganisms content of compost tea.	Znaïdi et al. 2002
<i>Fusarium</i> spp.	Spent mushroom substrate, pig manure, biogas residues.	Compost	Tomato and pepper	Compost application suppressed the pathogens between 20% and 90%.	Microbial population and activity.	Meng et al. 2018

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### **Source of the graphics**

#### *Front cover:*

Gallo-Roman harvesting machine, called Vallus. Source: U. Troitzsch - W. Weber  
(1987): Die Technik : Von den Anfängen bis zur Gegenwart

#### *Rear cover:*

Portrait of Columella, in Jean de Tournes, Insignium aliquot virorum icones.  
Lugduni: Apud Ioan. Tornaesium 1559. Centre d'Études Supérieures de la  
Renaissance - Tours



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## **Lucius Junius Moderatus Columella**

(AD 4 – 70) is the most important writer on agriculture of the Roman empire. His *De Re Rustica* in twelve volumes has been completely preserved and forms an important source on agriculture. This book was translated to many languages and used as a basic work in agricultural education until the end of the 19<sup>th</sup> Century.